Debrecen University
Centre for Agricultural Sciences
Faculty of Agriculture

4th International Plant Protection Symposium at Debrecen University

(11th Trans-Tisza Plant Protection Forum)

Recent developments of IPM

PROCEEDINGS

Editors:
György J. Kövics – István Dávid

18-19 October, 2006
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(The taxonomic status of the most important cryptic species of *Chrysoperla carnea* complex in Spain)

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Chrysopids have long attracted the attention of the applied entomologist for they are good candidates for use in IPM programs. They are distributed worldwide, have a wide host plant and prey range (Principi and Canard, 1984), can be easily mass cultured (Ridgway et al., 1980), manipulated using food sprays (Hagen and Tassen, 1980) and overwintering boxes (McEwen et al. 2000), and pesticide tolerant populations have been identified (Grafton-Cardwell and Hoy, 1985). Due to these characteristics in response to a survey in 1992, members of the Association of Applied Insect Ecologists ranked *Chrysoperla* ssp., the most important lacewings as unrivaled on the list of commonly used, commercially obtainable predaceous natural enemies (Tauber et al., 2000). Although a lot of work has been carried out on Chrysopidae, but regarding the many gaps in their natural history green lacewings are little known insects, and even their taxonomic status – at least that of the most important taxon *Chysoperla carnea* (Stephens) – is uncertain.

The systematic status of *Ch. carnea* has been changing, and instead of a polymorphic single species, a complex of sibling or cryptic species, the *Chrysoperla carnea* complex or *carnea*-group (Thierry et al., 1992; Thierry et al., 1998; Henry et al., 2001) should be now considered whose members` systematic status is not known enough (Tauber et al., 2000, Henry et al., 2001). Several attempts of multiple approaches such as courtship sonification (Henry, 1983, 1985), genetic studies with multilocus electrophoresis (Cianchi and Bullini, 1992), nucleotide sequences of COII, cytochrome oxidase I, cytochrome b and the large ribosomal subunit of the mtDNA (Lourenço et al. 2006), morphological characterization of adults and larvae (Thierry et al., 1992), ecophysiological variability (Thierry et al., 1994; Canard et al., 2002) have been made.

They supported the existence of various cryptic species among which one can find:

1) *Ch. carnea* former *Chrysoperla kolthoffi* (Navás, 1927) sensu Cloupeau (*Cc4* as song species), or "motorboat"(as song type) (Henry et al., 2002) or *Ch. affinis* former *Ch. kolthoffi* (Thierry et al., 1998); 2) *Chrysoperla lucasina* (Lacroix, 1912) (Henry et al., 2001) and 3) *Chrysoperla carnea* sensu stricto (Thierry et al., 1998) or *Cc2* ("slow-motorboat") or *Chrysoperla pallida* sp. nov. (Henry et al., 2002); 4) *Chrysoperla agilis* sp. nov. (Henry et al., 2003) or *Cc3* (Maltese).

In spite of the efforts made for clearing the taxonomic status of Palearctic *Ch. carnea* the present situation of species demarcations cannot be called satisfying because of the lack of agreement in reliable criteria (Tauber et al., 2000; Henry et al., 2001, 2002; Canard et al., 2002; Canard and Thierry personnel communication (= p.c.), 2003). There is a deep controversy between the two main groups of researchers (one of them uses mainly the substrate-born vibrational songs and certain morphological characteristics like shape of the male genital "lip" and "chin", another prefers ecophysiological traits and subtle morphological differences (like shape of the basal dilatation of the metatarsal claw, pigment distribution of the stipes, etc.) for distinguishing the cryptic species of *carnea*-group) because the first group concluded that the true *Ch. carnea* described by Stephens in 1836 and to be found in The Natural History Museum, London, must be *Cc4* (Henry et al., 2002) which according to the other group is another species, the *Ch. affinis* (Canard, 2003 p.c.). The other candidate species for being the "true" *Ch. carnea* may be *Cc2* mentioned above like *Ch. carnea* s. str. (Canard, 2003 p.c.) but in contrast with it, this taxon was assigned a new name, *Chrysoperla pallida* sp. nov. by Henry et al.,(2002). Regarding the lack of perfect evidences and the somehow too complicated illogical argumentation about the consideration of the decisive traits, the validity of these names, however, has not been discussed and accepted by the neuropterist community yet.

These are the theoretical or taxonomic troubles. But which are the practical ones? It should not forget the natural enemy role of *Ch. carnea*, because this taxon has been used in green houses and in the fields and orchards. It has been reared, tested, qualified and sold worldwide. It is a species about which a great deal of articles have been written. Main questions: Which taxon was the object of these studies? Which taxon can we buy at Koppert or Biobest? Which taxon's natural populations help growers in various countries? Regarding the study of the carnea group, there are considerable gaps almost everywhere: in Europe, America, Asia, Australia and Africa. We know only very little about the presence, distribution, ecological demands, preferences, habitats of the taxon which formerly was called as *Chrysoperla carnea*. However, there are some countries where, due to the work of few neuropterists, the natural common green lacewing populations have somehow been characterized. These

countries are France, the USA, Switzerland, England, Germany, Belgium, Romania and Hungary. Reducing our examination only to the European continent, it is a fact that the not mentioned European countries' common green lacewings represents white spots on the map of our knowledge. The following study shows the first steps of common green lacewing research in Spain.

Olive moth (*Prays oleae* (Bernard) is one of the most important insect pests of olive groves in the Mediterranean basin and so is in Spain, and Andaluzia (Spain is the biggest olive oil producer in Europe) as well. The second generation females lay eggs on the small fruits in early summer, and the emerging larvae bore within the olive fruit causing spectacular fruit drop in July and August (Ramos et al., 2005). Various methods are used against the moth population but in most cases insecticides are applied (Ramos et al., 2005). Taking into consideration the environmental and human feeding risks the development of integrated or biological control methods would be necessary for the environmentally friendly or organic production of olives. According to local observations the common green lacewing (*Chrysoperla carnea* (Stephens) sensu lato) may be an efficient predator of the olive moth eggs and caterpillars (Al-Asaad, 2004). However, which sibling species is the really efficient taxon?

In some years when the density of lacewings is proper, the natural control is efficient. However, in other years the density is small, and there is no natural control by lacewing larvae (Ramos et al., 2005). That is why the following questions can be raised: How is it possible to forecast the lacewing density? How can we improve the density of natural populations?

Possible solutions:

- identification of the lacewing species (sibling species) controlling olive moth caterpillars,
- measuring the predatory performance of lacewing larvae using *in situ* observation and laboratory experiments,
- study of population dynamics of lacewings and its dependence on major environmental factors
- determination the chrysopid fauna of some Andaluzian olive plantations and characterization of their population dynamics,
- measuring the efficiency of food sprays and over-wintering boxes for possible augmentation and conservation of common green lacewing adults,
- studying the impact of uncultivated areas for natural lacewing populations, mainly for their maintenance, over-wintering and distribution.

On the basis of these data it will be possible to develop a conservation and augmentation strategy for the natural populations as well as to select the best fitted species for the control and the probable rearing procedures. The possible utilization (a biological control technology can be developed for Spanish olive producers) of results will help to reduce the plant protection

charges in olive production and diminish the environmental contaminations by pesticides in the end products. With all these activities we can considerably contribute to the production of healthier and better quality food and also to the maintaining of declining biodiversity at European level.

Materials and Methods

Ch carnea s. l. adults were collected in 2003 and 2004 in olive groves (growing area) in Jaén and Granada counties and in 2005 in the park and adjacent orchards of Granada (in the southeast of Spain). Captures were obtained by chromatic sticky traps (yellow and blue), olfactory traps, light traps and sweeping net. Individuals were identified according to the descriptions of Thierry et al., (1992), Canard, M. (2002 and 2003, pers. comm.), Duelli, P. (1995 and 1999, pers. comm.) and also samples of various morphological types (courtesy of Thierry, D.) and song morphs (courtesy of Duelli, P.) have been used. In case of *Ch. agilis* and *Ch. affinis* atypical specimens were excluded. The individuals captured by sticky traps and light traps were collected, put into ethanol and identified several weeks later. In case of the sweeping, living specimen were identified immediately after catching. Table 1 contains the basic data of sampling.

Table 1. Basic data of collection in southern Spain

Site	**Habitat**	**Year**	**Catching method**	**Number of individuals caught**
Ubeda	olive grove	2003	coloured sticky traps	207
Mancha Real	olive grove	2004	coloured sticky traps	203
La Nava	olive grove	2004	coloured sticky traps olfactory traps	367
Láchar	olive grove	2004	coloured sticky traps	63
Fuerte del Rey	olive grove	2004	coloured sticky traps	24
Granada	park, mixed orchards	2005	sweep net	76

Results and Discussion

Abundance values of the species caught in southern Spain are presented in Table 2.

Table 2. Number and proportion of sibling species of common green lacewings in Andaluzia

Sites	***Ch. agilis* ind. (%)**	***Ch. carnea* s. stricto ind. (%)**	***Ch. lucasina* ind. (%)**	***Ch. affinis* ind. (%)**	***Ch. carnea* s. lato ind. (%)**	**Total**
Ubeda 2003	125 (60.4)	44 (21.3)	25 (12.1)	9 (4.5)	4 (1.9)	**207**
Mancha Real 2004	165 (81.3)	17 (8.4)	13 (6.4)	4 (1.9)	4 (1.9)	**203**
La Nava 2004	287 (78.2)	11 (3.0)	14 (3.8)	1 (0.3)	54 (17.7)	**367**
Láchar 2004	57 (90.5)	-	1 (1.6)	-	5 (7.9)	**63**
Fuerte del Rey 2004	20 (83.3)	1 (4.2)	1 (4.2)	-	2 (8.3)	**24**
Granada 2005	71 (93.4)	1 (1.3)	1 (1.3)	2 (2.6)	1 (1 .3)	**76**
Total	**725 (77.1)**	**74 (7.9)**	**55 (5.9)**	**16 (1.7)**	**70 (7.4)**	**940**

Ch. agilis predominates without question with its 77% value. It is followed by *Ch. carnea* s.str. (8%), *Ch. lucasina* (6%), *Ch affinis* (2%). *Ch. carnea* s.l. represents the undeterminable individuals whose identification because of their morphological injury, the great quantity of unremovable glue remains on their body or the considerable variability of their characteristic traits was not possible (Table 2).

The four sibling species of the *Ch. carnea* complex have been found mainly in the olive groves of Andaluzia. The species with the highest number of individuals collected was *Ch. agilis*. It is one of the sibling species that is the most difficult to diagnose from preserved or alive caught individuals. As to their identification on the basis of vibrational patterns, any of the European neuropterists – with the exception of a minority (mostly American) of searchers creating the methodology – did not have the

facilities and opportunities to verify them. *Ch. agilis* is easily confused with the most often occurring species of the *carnea* group, *Ch. affinis* and *Ch. carnea* s.str. In addition, there is another possibility for confusion as these dominant European lacewing taxa are named differently (see above). Besides the systematic difficulty also their distribution and occurrence have not been studied properly. The descriptors of the species analyzed 74 individuals whose origin is shown in Table 3. Our results based on 940 lacewings, the biggest number of *Ch. carnea* complex specimens ever identified in Spain. According to these data and those of Henry et al., (2003), the occurrence of *Ch. agilis* is common in southern Spain, in the Mediterraneum and the species occurs in Central-Asia (Iran) as well. Besides *Ch. agilis*, *Ch. lucasina,* another rather Mediterranean or Atlanto-Mediterranean lacewing (Henry et al. 2002, Bozsik et al., 2003) and *Ch. carnea* s.str and *Ch. affinis*, two in the mainland Europe dominant species (Thierry et al. 1996, Bozsik et al., 2003) have been collected. Regarding the number of captured specimens, it seems that *Ch. agilis* is the dominant species whose impact on olive moth caterpillars can be the greatest. The abundance and frequency (1.7%) of *Ch. affinis* was the smallest, and the other sibling species with their 6-8% frequency can have only less significant role in biological control of *P. oleae*.

Table 3. Collection sites of *Ch. agilis* (on the data of Henry et al., 2003) (No data = the sites were indicated as collection places but the number of collected specimens was omitted)

Local site	Country	Date	Number of individuals
Azores archipelago	Portugal	August 2000	16
Southern Spain (Alicante, Granada)	Spain	July 2001	4
Southern France (Carcès)	France	August 1994	5
The Alps (Ticino)	Switzerland	1981-94	10
Southern Italy and Sicily	Italy	July 1993	21
Malta	Malta	1991	4
Xilokastron, Kalentzi and other sites	Greece	June 1994	10
Eilat	Israel	October1993-94	4
Northern Iran	Iran	June 2002	No data
Agadir	Morocco	1985	No data
Total number of individuals			74

Considering the planned research activity indicated in the introduction, all the studies mentioned there should be done firstly on *Ch. agilis.*

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FIRST DATA ON THE SIBLING SPECIES OF THE COMMON GREEN LACEWINGS IN SPAIN (NEUROPTERA: CHRYSOPIDAE)

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Summary

Common green lacewings are good candidates for use in IPM programs because they are distributed worldwide, have a wide host plant and prey range, can be easily mass cultured, manipulated using food sprays and overwintering boxes, and pesticide tolerant populations have been identified. Although a lot of work has been carried out on Chrysopidae, but regarding the many gaps in their natural history, green lacewings are little known insects, and even their taxonomic status – at least that of the most important taxon *Chysoperla carnea* (Stephens) – is uncertain. It is instead of a polymorphic single species, a complex of cryptic species, the *Chrysoperla carnea* complex or *carnea*-group. In present contribution composition of the natural *Ch. carnea* population was investigated in order to establish systematic bases for biological control studies in olive groves of Spain. Our results based on 940 lacewings, represents the biggest number of *Ch. carnea* complex specimens ever identified in Spain. *Ch. agilis* predominated with its 77% value. It was followed by *Ch. carnea* s.str. (8%), *Ch. lucasina* (6%), *Ch affinis* (2%). Regarding the number of captured specimens, it seems that *Ch. agilis* is the dominant species whose impact on olive moth caterpillars the greatest can be. The abundance and frequency of *Ch. affinis* was the smallest, and the other sibling species with their 6-8% frequency can have only more modest role in biological control of *P. oleae*.

INTEGRATED MANAGEMENT OF THE DUTCH ELM DISEASE IN ALHAMBRA AND GENERALIFE FORESTS (GRANADA, SPAIN)

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Dutch elm disease, caused by the ascomycete *Ophiostoma ulmi* Buisman, has been responsible for the disappearance of hundreds of millions elms trees in the northern hemisphere (Brasier, 1990, 1991; Brasier and Mehrotra, 1995). Since its appearance, at the start of the century, it has produced two pandemics. The first of these began in North-Eastern Europe around 1910 and the second owed its appearance -around 1940- to two races of greater pathogenicity (North-American NAN and Eurasian EAN), causing the original agent to be replaced by the newly named *Ophiostoma novo-ulmi* Brasier.

The geographic isolation of Spain has resulted in a considerable delay in the arrival of the agressive strain (NAN) of the elm disease, which only appeared from 1980 onwards (Gil Sánchez, 1990). Due to the rapid expansion of the disease, by 1990 healthy elms were already considered rare. In the Granada province, elm infection appeared from 1992 onwards, and the province is cited amongst the last affected by the disease in Spain (González-Ruiz, 1990, 1995; González-Ruiz and Prieto Fernández, 1995).

The pathogenicity of *O. novo-ulmi* is caused as a result of the penetration and development of the hyphae in the vessels of the xylem, which generate thousands of conidia with blok the flow of water, as well as by the produced toxins, which cause progressive dessication and death of the tree (Ipinza Carmona et al, 1990).

As it is commonly known, the fungus is poorly adapted for any other type of transport (Pajares Alonso, 1990), and it requires the participation of species of Scolytidae family: *Scolytus multistriatus* and *Scolytus scolytus*, which possess specific tegument adaptations for the transport of the fungal spores, and consequently are well adapted to infect new healthy elms during their feeding on the tree canopy during its dispersal flight. They reproduce

in weakened trees, and in sick branches, where they construct their reproductive galleries. When the newly emerged adults begin their flight dispersal, destined to seek out appropriate material for their reproduction, they fed in twig feeding galleries on the forks of the canopies, thus causing the formation of further infections.

The development of the infection causes progressive debilitation and death of the affected elm, whilst the process of root grafting between trees, accelerates the transmission of the disease over a short distance.

The presence of the scolytid beetle's reproductive galleries in the dead or ill trees, results in exponential growth of the disease in the affected elm, increased by the transport and storage of the resulting timber.

DEFELOPMENT OF THE CONTROL PROGRAMME

Once the NAN aggressive strain was detected in the Alhambra and Generalife forest, at the Spring of 1994, a Programme of integrated management was rapidly set up, with the objective of reducing levels of infection, and containing the expansion of both pathogen and insect vectors (González Ruiz et al, 1998). The success of this programme mostly depended on the adequate co-ordination of all its elements (Lanier, 1990; Pajares Alonso and Martínez de Azafra, 1990), which will be now described.

I. Elms inventory.

The first of the control elements involves the formation of an inventory which will permit the identification of all the elm trees and which will provide information on their characteristics (diameter, height), and phytosanitary state. To get an easier handling of data, four different areas have been considered : 1/ "Alamedas de Gomérez" wood, 2/ "Generalife" woods, 3/ "Alhambra" gardens, and 4/ "San Pedro" woods. In each area, elms were labeled and represented on a plan to the scale of 1: 300. Periodic observations of the phenology of the elms are made, to adequately adapt the different elements of the control programme. An initial number of 1955 elms (Figure 1) have been catalogued, the majority of them from the Generalife and "Alamedas de Gomérez" woods, and a smaller proportion from the S- Pedro woods and Alhambra woods. Considering the notable elms, the majority are found in the "Alamedas" zone (Figure 2).

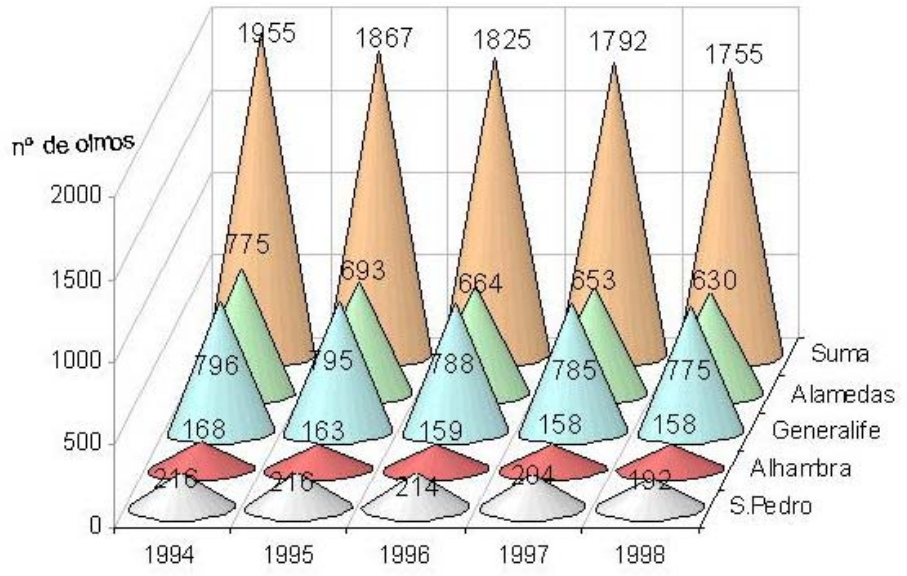

Figure 1. Elm abundance and distribution during the period of control of DED in Granada forests

II. Inspections

Early detection of infections is achieved by means of a careful inspection of the tree canopies, from the different angles (Cannon et al., 1985). The possibility of being able to intercept the infection is improved the earlier it is detected. To this, the use of cranes is important, to give better access to hidden areas.

A total number of 396 trees have been affected in the period 1994/1998 (which represents 20,2% of the total number of trees) of which 141 in 1994; 60 in 1995; 43 in 1996, 68 in 1997 and 84 in 1998. The number of infected elms as a percentage of the total number of trees was 7.2% (1994), 3.2% (1995) ; 2.4% (1996), and 3,8 (1997) and 4.8% (1998).

The highest level of incidence was observed in the notable elms (initial number was of 523) in which 165 examples (Figure 3) were affected. In these elms, the annual levels of infection were 17% (1994), 6,7% (1995) 2,3% (1996), 3,5 (1997) and 4,2% (1998). The statistically significant reduction was observed in the four areas during the study.

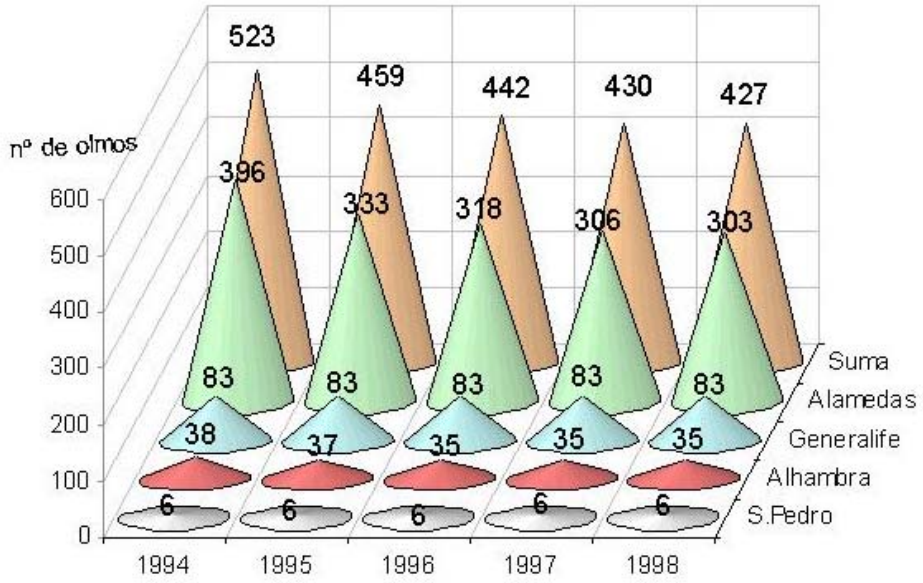

Figure 2. Notable elm abundance and distribution during the period of control of the DED in Granada forests

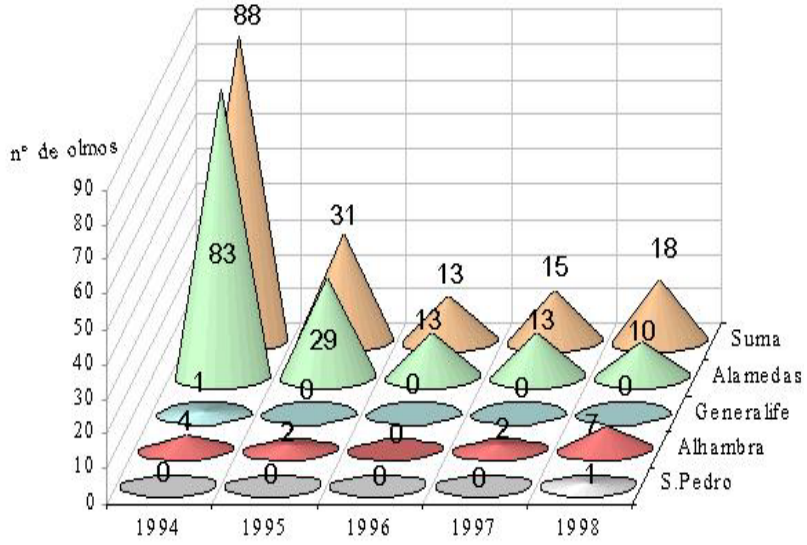

Figure 3. Distribution of notable elm affected by DED during the control programme.

III. Therapeutic pruning.

Their objective is the eradication of the pathogen by removing infected parts of the tree (Hart, 1970). The success of the process is inversely proportional to the size of the lesion in the xylem, hence the necessity to make periodic inspections. Cranes are used to give access to the canopies, as well as manual climbing techniques. The infected branch is removed. The number of elms treated was 183, of which 84 in 1994, 24 in 1995, 6 in 1996, 33 in 1997 and 36 in 1998.

Therapeutic pruning has resulted in the recovery of 55, 19 and 4 elms affected in 1994, 1995 and 1996 respectively, nevertheless, the success of therapeutic pruning has varied between 80% (infection less than 10%) , to 40% (infection between 10 and 20%) and 33% (infection greater than 20%).

When level of infection was greater than 20% of infected foliar surface, the pathogen had progressed as far as the vascular bundles of the trunk xylem. Initially (1994), the removal of the greater part of the canopy and a variable length of the trunk of these affected trees, were carried out, but in no case has this process resulted in the recovery of the tree. In fact it is increased the risk of root transmission to neighbouring elms.

IV. Limiting root transmission.

The transmission of the pathogen trough root grafts is quite common way of infection (Verral and Graham, 1935), being in our program responsible for at least 20% of mortality in 1995, 70% in 1996, and 40% in 1997 It was especially prevalent in “Alamedas de Gomérez” zone, where it was responsible for 25%, 90% and 100% of mortality (1995, 1996 and 1997 respectively).

a. Mechanical disruption of xylem vessels. A ring shaped incision is made at the base of the trunk which affects the last rings of the xylem, leaving the pathogen confined to the aerial part of the elm.

b. Chemical destruction of the root. The triclorade pirinidil oxi-acetic acid was added in the incision. Satisfactory results were observed in most of cases.

c. Mechanical destruction of the bridge-roots. The destruction of root connections between elms is made by digging trenches manually or mechanically, thus attempting to isolate different groups of elms or individual trees. According to the depth of theses trenches, the effectiveness ranged from 70% to 100% when depth was increased from 60 cm to 1.5 m.

V. Control of vectors.

In general lines, the scolytid elm bark beetles represent the main transmission vehicle of the disease. Several measures have been used in

order to reduce the scolytids populations, and therefore, the frecuency of aerial transmission of *O. novo-ulmi*.

a. Sanitation

This forms the principal element of vector control and consist of removal of the material used for their reproduction (Van Sikle and Stern, 1976). Its importance becomes clear when we are into account, the fact en adult elm may produce up to 500.000 individuals.

On those elms in which more than 25% of the canopy is affected, the beetle's penetration holes, which correspond to their subcortical gallery systems, are frequently observed. However, due to the secondary character of these insects, every elm debilitated, either by desiccation, (suffered until the end of 1995) , or as a result of work carried out on its surroundings, becomes an optimal material for the beetles reproduction, despite the fact that it doesn't show visible symptoms of the disease. in other causes the existence of cavities and rot, aggravated by excessive humidity, cases the proliferation of other pathogenic fungi such as *Armillaria mellea* (responsible for white rot in roots), as well as an increasing frequency of colonies of termites, which together cause cracking in the branches and trunk. This debilitations provide an excellent substrate for scolytid beetle reproduction. It is therefore necessary to fell these tress, When their size and position permit, this is done directly from the base. However, more frequently, in the case of larger, denser elm, they are gradually cut down and chopped up later. During the time to complete this process, the remains of the trees were provided with informative posters. Later, the remains of the tree are removed as quickly as possible and incinerated. When there were fire danger the timber was subjected to treatment with insecticides and later covered with plastic to improve the efficacy of the treatment. After felling, it is necessary to considerably reduce the height of the stump because this might hold vital reservoirs of the pathogen.

The clearing up process in the Alhambra resulted in the felling a total number of 265 elms, which represents a 13,5% of the original population. Of these, 88 elms corresponded to 1994, 43 to 1995, 36 to 1996, 30 to 1997 and 68 to 1998. The percentages being 4.5% in 1994, 2,3% in 1995, 2% in 1996 and almost 1,6% in1997 and 3,6% in 1998.

However, considering only notable elms, the number of felled trees was of 19,6% from the original population. From these, 64 trees were felled in 1994, 17 in 1995, 12 in 1996, 3 in 1997 and 7 in 1998. The corresponding mortality percentages being 12.2%, 3,7% 2,7%, 2.3 and 1.6% during the study period (Figure 4).

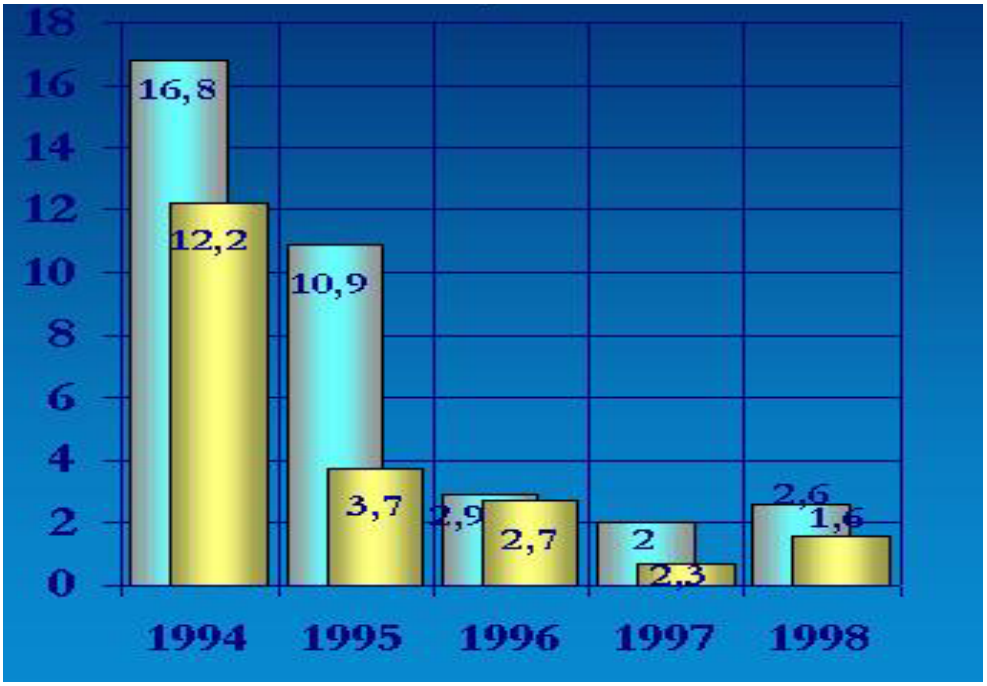

Figure 4. Percentages of notables elms afected (whyte) and killed (yellow) by DED during the period of application of control programme.

b. Pheromone Traps

A technique applied frequently in population monitoring of bark beetles is the installation of flight interception traps containing synthetic aggregation pheromones (Wood, 1982). For *Scolytus multistriatus* the pheromone consists of a three fold mixture of *a* -multistriatin, *4-metil-3-heptanol* and *a* -alfacubebene. A total number of 8 traps (close to 1/ha) have been installed on the periphery of the and suitably distanced from the tress. These traps, periodically examined, showed the variation in densities of flying beetles, and reveal the presence of close-by focus of dispersion, and permitted to compare the population sizes in different years (Figures 5, 6, 7 and 8). Three complete generations and a partial fourth generation have been observed, in accordance with the observations of Pajares Alonso (1987) in central Spain. Generation length was 55 to 60 days for the first generation and 45 to 50 days for the second and third generations. The number of captured individuals was 3272 in 1995 and 4418 in 1996, although this increase was not statistically significant different.

In the case of *Scolytus scolytus*, whose commercial pheromone doesn't had a great attraction capacity, the pheromone was used together with lures of trunks of recently felled elms, protected by an insecticide, as

well as the neighbouring tress. The volatile chemicals given off by the trunk-lures improved the level of attraction of the synthetic pheromones.

c. Biological control of the elm bark beetles

A programme of biological control has also been applied, achieved by mass rearing chalcidoidea parasitoids of *S. multistriatus*, (*Cheiropachus quadrum, Raphitelus maculatus, Dendrosoter protuberans* as main species) was carried out in an insectorium, by using the olive bark beetle *Phloeotribus scarabaeoides* (Bernard.) as substitution host. For the mass rearing of parasitoids, live tree timber is infested with bark beetles. Estimates of the number of released individuals have been approximately 36.000 and 43.000, being the estimated offspring is 24.000.000 individuals which corresponds to the number of beetles normally produced in 50 notable elms. The test carried out throughout 1996 showed levels of parasitism greater than 17%, in respect to the 6,5% observed in material obtained in 1994, a statistically significant increase (Figure 9).

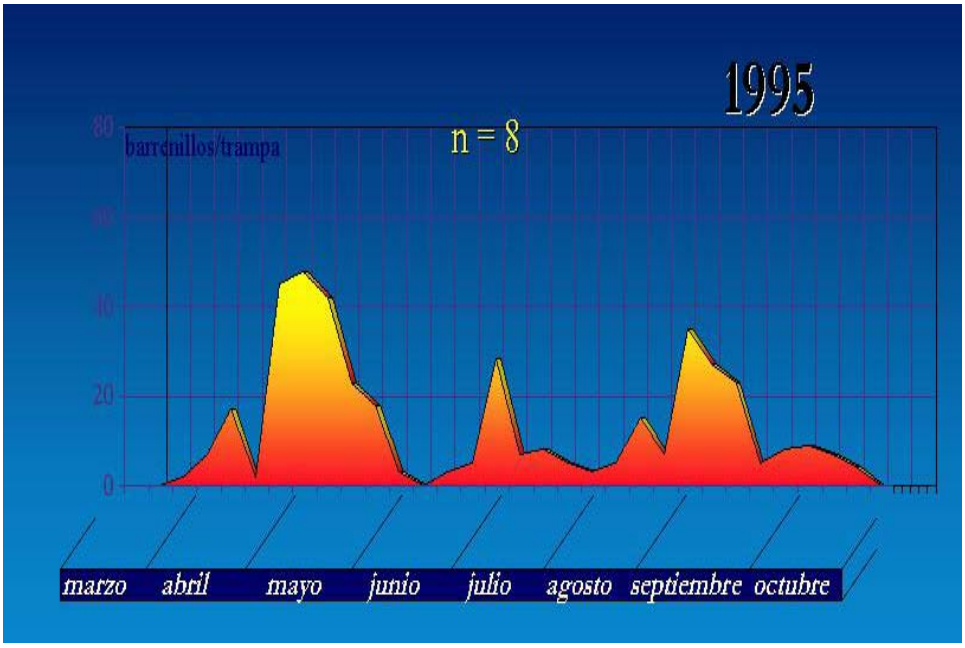

Figure 5. Pattern of flight (no. of ind. per trap) of *Scolytus multistriatus* during 1995

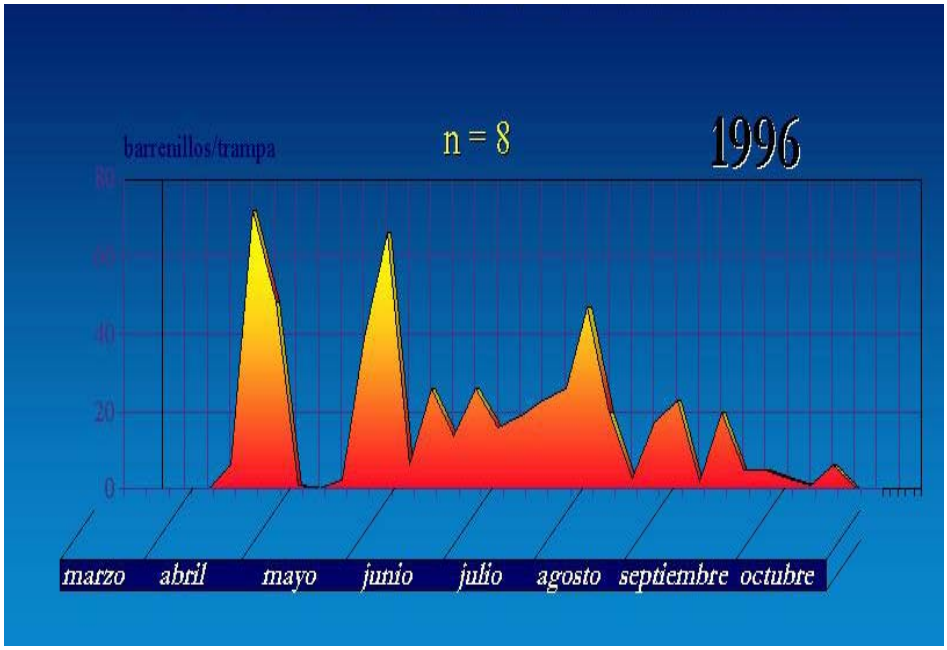

Figure 6. Pattern of flight (no. of ind. per trap) of *Scolytus multistriatus* during 1996

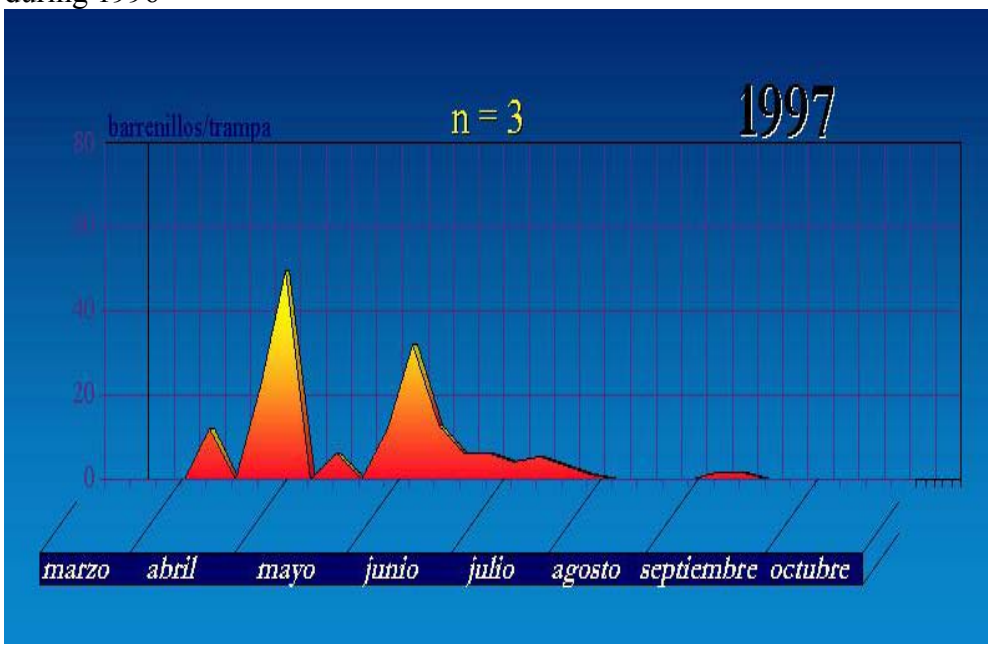

Figure 7. Pattern of flight (no. of ind. per trap) of *Scolytus multistriatus* during 1997

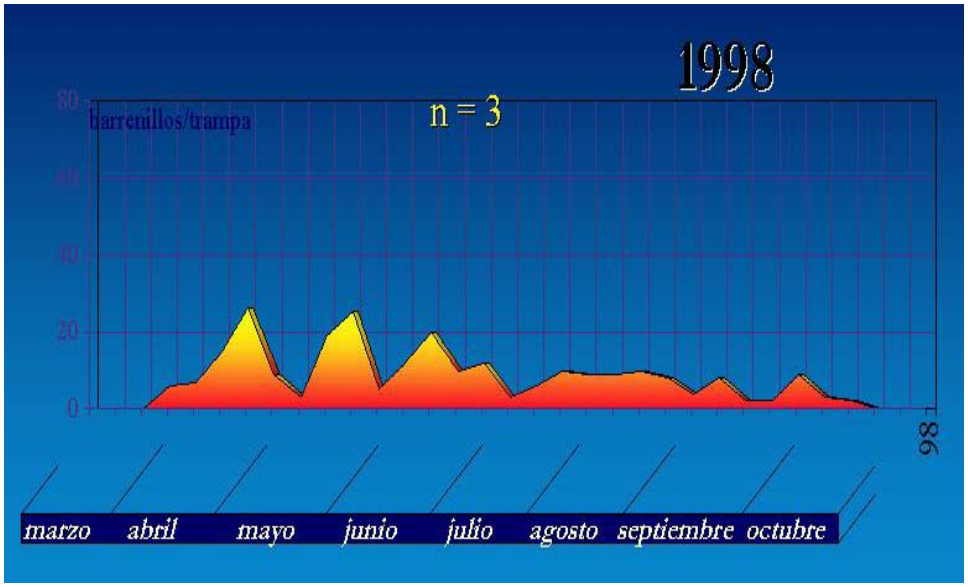

Figure 8. Pattern of flight (no. of ind. per trap) of *Scolytus multistriatus* during 1998

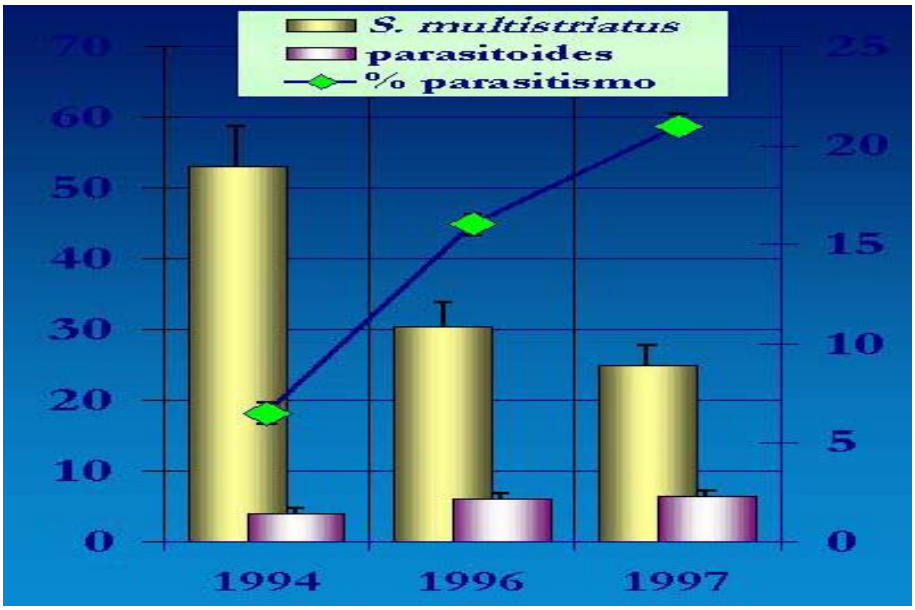

Figure 9. Evolution of the density of emergence of *S. multistriatus* and level of parasitization after mass release of its parasitoids in Alhambra and Generalife elm woods

d. lnsecticide application

In spite the last element, it was absolutely essential to protect the canopies of elms by means of insecticides application (Lanier et al., 1984; Pajares Alonso and Arévalo, 1987). The system employed consisted of the application of a piretroid insecticide *a*-cypermetrin 0.3% or lambda-cyhalotrin.0.7%) achieved by spraying from the ground and sometimes from the air. The applications were made during absolutely calm atmospheric conditions to ensure maximal cover. The application of the insecticide was carried out, taking into account the phenology of the elms, the phenology of the beetles, and periods of rain, attempting to achieve an adequate and regular temporal distance between them.

VI. Injection of Fungicides (Tiabendazol)

Systemic chemical treatments by means of injection of fungicides was also carried out. Because of the high cost and inconveniences of this type of treatment, it was only been used in less than 10% of the notable elms. The application was used as a preventive measure in 50%, 97% and 100% of cases in years 1994, 1995, and 1996. Following the evolution of the elms injected with tiabendazol has allowed us to verify its ineffectiveness in cases with a high proportion of affected foliar surface (elms lopped during 1994), as well as cases infected later via the roots. Moreover, some of the preventive injected elms (1 injected in 1995, 3 elms in 1996), have also show symptoms of foliar infection, which indicated its relatively low efficacy as described by Shigo and Campana (1977).

VII. Induction of elm resistance by *Verticillium dahliae* inoculations

With the objective of determining whether other pathogenic fungi would be effective at inducing a resistance mechanism in the elm under ecological conditions similar to whose in the Alhambra and Generalife, and with the objective of possibly incorporating them into a control programme, a trial was carried out during the summer of 1996 (Gázquez Alcoba and González Ruiz, 1998). Inside a study area, 4 subgroups of 20 elms were established and each of these groups was subjected to a different treatment:

Group A (distilled water, control group)
Group B (*0. novo ulmi*),
Group C (*Verticillium dahliae* WCS 859)
Group D (*0. novo ulmi* and *Verticillium dahliae* WCS 859).

The method used in the application of the biocontrol agent, crucial for its efficacy, consisted of chiselling with a pouge, in accordance with the technique supplied by Heidemij Realisatie (Aperdoorn, Netherlands).

The results obtained over the summer of 1996 reveal large differences between the symptoms of the elms in the different groups (Figure 10.). A total absence of symptoms was observed in the elms in the control group (group A), in those injected with *V. dahliae* (Group C) and in the injected with *0. novo-ulmi* and *V. dahliae*.(Group D).

As was expected, an increase level of mortality occured in the elms infected with *0. novo-ulmi* (Group B). A later examination of the elms carried out during the summer of 1997, clear the lack of effectiveness of the treatment with *V. dahliae*, as it was expected.

The efficacy of treatment was therefore only maintained during a temporary of the elms, making it necessary to renew the treatment every year to induce resistance. Despite this, these results were highly satisfactory, and opened up important routes for the improvement in the efficacy of integrated control.

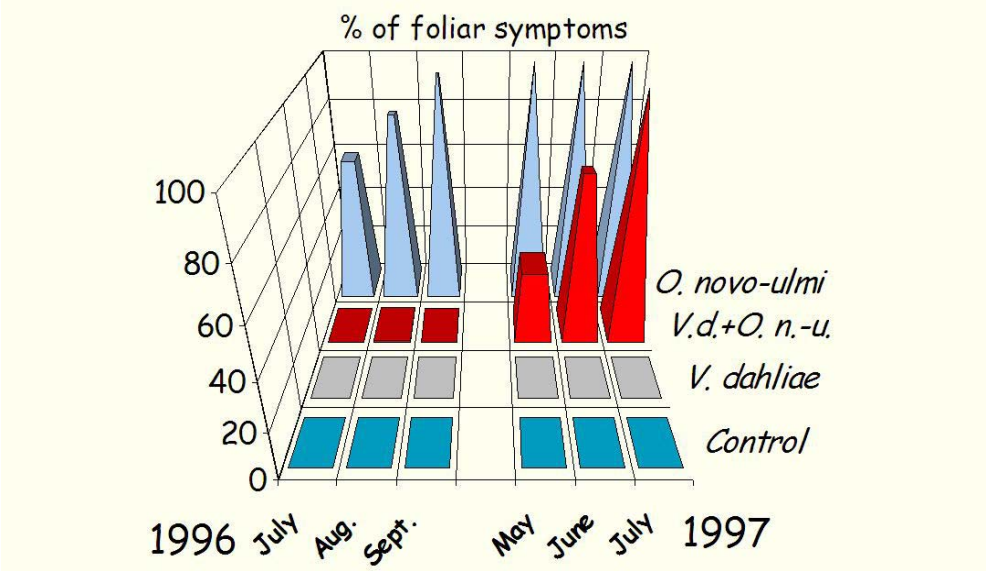

Figure 10. Elms evolution (foliar symptoms) during the experiment

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INTEGRATED MANAGEMENT OF THE DUTCH ELM DISEASE IN ALHAMBRA AND GENERALIFE FORESTS (GRANADA, SPAIN)

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Summary

Dutch elm disease, caused by the ascomycete *Ophiostoma ulmi* Buisman), has been responsible for the disappearance of hundreds of millions elms trees in the northern hemisphere. Since its appearance, at the start of the century, it has produced two pandemics. The first of these began in North-Eastern Europe around 1910 and the second owed its appearance -around 1940- to two races of greater pathogenicity (North-American NAN and Eurasian EAN), causing the original agent to be replaced by the newly named *Ophiostoma novo-ulmi* (Brasier). The geographic isolation of Spain has resulted in a considerable delay in the arrival of the agressive strain (NAN) of the elm disease, which only appeared from 1980 onwards. Due to the rapid expansion of the disease, by 1990 healthy elms were already considered rare. In the Granada province, elm infection appeared from 1992 onwards, and the province is cited amongst the last affected by the disease in Spain. The pathogenicity of *O. novo-ulmi* is caused as a result of the penetration and development of the hyphae in the vessels of the xylem, which generate thousands of conidia with block the flow of water, as well as by the produced toxins, which cause progressive desiccation and death of the tree. As it is known, the fungus is poorly adapted for any other type of transport, and it requires the participation of elm bark beetles (Scolytidae): *Scolytus multistriatus* and *Scolytus scolytus*, which possess specific tegument adaptations for the transport of the fungal spores, and consequently are well adapted to infect new healthy elms. The development of the infection causes progressive debilitation and death of the affected elm, whilst the process of root grafting between trees, accelerates the transmission of the disease over a short distance. The presence of the scolytid beetle's reproductive galleries in the dead or ill trees, results in exponential growth of the disease in the affected elm, increased by the transport and storage of the resulting timber. The paper presents the heroic, many-sided fight (chemical, biological control) of the country's specialists against the disease and its vector in order to save the elm forests of Alhambra and Generalife.

RESEARCH AND DEVELOPMENT IN PESTICIDE CHEMISTRY
CURRENT STATUS AND A GLIMPSE AT THE FUTURE

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Natural, synthetic and semi-synthetic crop protection chemicals and disease control chemicals (altogether pesticides) have been one of the key components of modern agriculture as well as of animal and human disease vector control over the past century and will undoubtedly be used for decades. For 2005, the global market of agrochemicals used for pest control was US$33.6 billion (AGROW, 2006a), which is a 45% increase over global sales in 1990. From 1990 pesticide export more than doubled to about US$ 16 billion in 2005 (FAOSTAT, 2006), reflecting the impact of international trade and globalization. These figures, however, do not truly reflect the important changes that have happened in chemical terms in this continuously evolving field.

Recent studies indicate that major agrochemical companies spend an average of 7.5% of their annual sales on research and development of new crop protection products and on improving the activity or safety of existing ones (CropLife, 2005; Phillips McDougall, 2005). The development of a new product from discovery to first sales typically takes 8-9 years and costs roughly US$200 million. Of this, ca. US$67 million is spent on chemistry-related research, ca. US$80 million on biological studies, and ca. US$53 million on toxicological and environmental fate studies. The following chemically oriented overview will focus on research and development of new pesticides emphasizing selected aspects.

Need for new compounds

The main driving forces behind the research for new pesticides are as follows:

a) improvements over or replacement of existing products by new compounds that are more environment-friendly and less toxic to non-target organisms;
b) resistance towards currently used control agents;
c) new pests and diseases, including invasive species, necessitating novel control methods;
d) economic incentives.

Of these, resistance is a challenging motive to develop new pesticides. The continuous reliance on one particular compound class in a field inevitably increases the risk of the development of resistance towards that particular compound type. This resistance can extend to other, structurally different classes having the same mode of action. Instances of resistance to at least one pesticide active ingredient have been recorded for over 540 of insect species (IRAC, 2005), for over 300 weed biotypes (Heap, 2006; HRAC, 2005), and for some 350 plant pathogen isolates (FRAC, 2005). While many of these cases are from laboratory findings, field performance failures have frequently been reported. Thus, resistance management has become an essential part of crop protection and one of the promising ways of decreasing the risk of its occurrence is the use of novel compounds, ideally with a different mode of action.

Ways to find new bioactive compounds

It has recently been estimated that some 100,000 compounds have to be tested to bring a new pest control agent to market. These new substances could be obtained by:

a) natural product screening;
b) screening of randomly prepared synthetic compounds, e.g., in-house or acquired compound libraries;
c) analogue design using structural modifications of natural products or compounds developed by other companies;
d) enzyme inhibitor design based on vital biochemical reaction mechanisms;
e) computer aided molecule design using the three-dimensional (3D) structure of the target site;
f) serendipity.

The following paragraphs will discuss some of the above points.

Natural product-based pesticides. Natural products are exceptional sources of structural diversity and of unique chemical scaffolds that, by virtue of their ecological role, have proven to be capable binding to target proteins. Although the currently used pest control agents are mostly synthetic compounds, there are several pesticides obtained from plants, microorganisms or animals (Ujváry, 2002, 2003). These sources are usually renewable, and conventional breeding or genetic engineering can yield strains that biosynthesize substances originally producing in economically unacceptable low yields. Moreover, directed biosynthesis using non-natural precursors (e.g. amino acid analogs) that are then incorporated

enzymatically into the final molecule can give rise to novel and complex structures that would hardly be attainable by chemical synthesis. Systematic screening of botanical or microbial extracts will certainly uncover novel bioactive compounds from time to time.

A few pesticides are semi-synthetic substances obtained by appropriate synthetic modifications of natural products. It is expected that the number of such semi-synthetic derivatives with highly complex structure and with optimized potency against pests which are not susceptible to the original natural product, will increase. Some representative examples of structurally diverse natural products and synthetic analogs derived from them are shown in Figure 1.

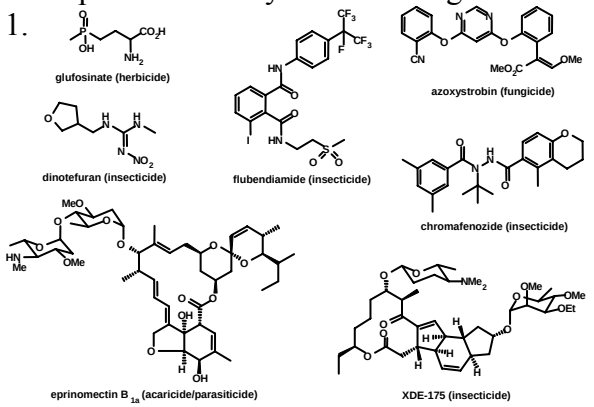

Figure 1. Structural diversity of selected natural products and other related pest control agents. For eprinomectin and XDE-175, only the major component of the commercialized material is shown.

For agricultural use, a natural product should be: a) efficacious against target species; b) safe and selective; 3) sufficiently stable in the field; c) standardized for composition and formulation; and d) readily available. If some of these criteria are not met, appropriate synthetic modifications or analogue design can lead to marketable pest control agents, as happened with the unstable natural strobilurins that served as models for a broad range of synthetic fungicides (e.g., azoxystrobin, Figure 1). Eprinomectin is one of the semi-synthetic derivatives of the parasiticide avermectin. Recently, fermentation provided complex core structures for semi-synthetic modifications affording XDE-175 (Figure 1), a second-generation version of the microbial insecticide spinosad (AGROW, 2006b; Sparks et al., 2001). It is expected that genetic engineering of complex biosynthetic pathways into tractable host (micro)organisms will be increasingly used to produce structurally complex chemicals on an industrial scale.

Discovery through screening of synthetics. Synthetic chemistry has long been a most successful tool in pesticide discovery. The origin of several

current pesticides can be traced back to projects initiated purely on chemical grounds, typically around structures of academic interest. Systematic bioassays of novel synthetics can result in a resurgence of interest in a forgotten mode of action. Thus, the discovery of imidacloprid revived research in the nicotinic acetylcholine receptor (Yamamoto and Casida, 1999) and within 15 years the neonicotinoids, exemplified by dinotefuran in Figure 1, captured over 15% of the insecticide market. A recently rediscovered insecticide site of action is the ryanodine receptor, the target of the botanical Ryania insecticide. The recent discovery of flubendiamide, a novel phthalic acid diamide (Figure 1), and the structurally related rynaxypyr, a new anthranilic acid diamide derivative, both acting at this receptor will certainly provide additional insecticides with novel mode of action (Nauen, 2006).
This traditional line of research is still worth to be pursued because it can be a source of new structures.

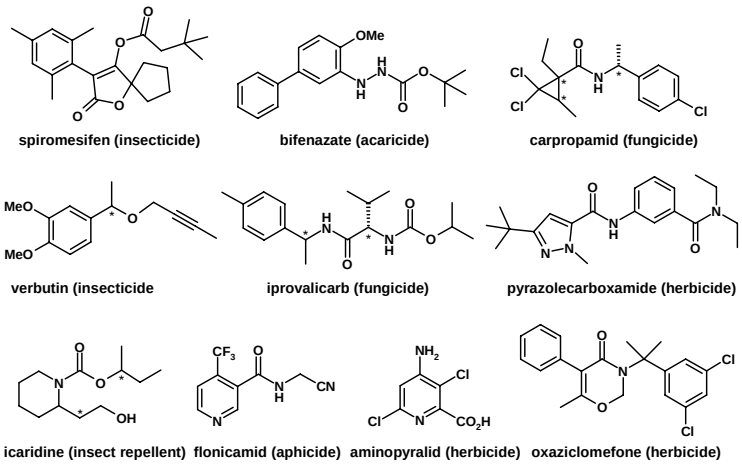

Figure 2. Chemical structure of selected pest control agents with novel structures. Asterisk (*) denotes chiral center.

Combinatorial chemistry. Combinatorial synthesis is an automated process to rapidly prepare large sets (libraries) of structurally related compounds by combining series of molecular building blocks. This technique generates, typically within days, hundreds or even thousands of analogs around core structures either in solution or on solid-phase. While early efforts were aimed at quantity to produce tens of thousand of closely related substances, often as unseparated mixtures, nowadays there is more emphasis on quality and diversity. While combinatorial chemistry has had a major impact on lead compound generation and optimization in the pharmaceutical industry, reports on its application in pesticide research are still scarce (Kleschik et al., 2003). The first successful example of the combinatorial approach was

the identification from a library of 8000 amides and esters of a pyrazolecarboxamide (Figure 2) having herbicidal activity at 100 g/ha (Parlow and Normansell, 1996). Recently, sets of 5-50 member libraries of acylaminoketone ecdysteroid receptor agonists, as analogues of dibenzoylhydrazine insecticides, afforded several compounds highly active *in vitro* (Garcia *et al.*, 2005). A broad-range, whole-organism pesticide screen of a diverse set of heterocycles, designed to possess physicochemical properties required for good uptake and translocation, afforded several fungicidal compounds (Martínez-Teipel et al., 2005).

High-throughput screening. High-throughput screening is a miniaturized and automated procedure in which a large number of compounds are screened rapidly and in parallel for readily measurable biological responses. In contrast to pharmaceuticals research where screening is typically done not on the target species, that is in humans, but in animal models and/or *in vitro* systems, pesticide research has the cost and risk saving advantage of testing candidate substances directly on target species *in vivo* thus minimizing problems due to unpredictable absorption and metabolism that are more or less absent in *in vitro* assays.

Molecular modeling and structure-based design. Computer-aided design of bioactive compounds using the 3D structure of small molecule ligands and target enzymes or receptors has become a reality during the past decade (Walter, 2002). However, there are only a handful of 3D structures of complexes between pesticidal ligands and relevant proteins (Table 1). These structures, usually obtained by X-ray crystallography, permit the examination of interactions between ligand and target in atomic detail and can also reveal novel binding sites. Importantly, 3D structural data allow screening *in silico* of millions of molecules (virtual compound libraries) within days.

The different binding of selective and non-selective compounds can also be studied *in silico* if the relevant target site structure is available. Comparison of the X-ray structures of plant (*Arabidopsis thaliana*) and rat 4-hydroxyphenylpyruvate dioxygenases complexing hydroxypyrazolyl ketones that are structurally related to the herbicides mesotrione and isoxaflutole revealed the structural basis for the selectivity of these herbicides (Yang et al., 2004). Recent binding mode studies of the cytochrome P450 inhibitor triazole fungicide metconazole utilized the crystal structure of fluconazole bound to bacterial lanosterol 14α-demethylase (Ito et al., 2004). In the field of insect control, the recent

Table 1. Representative examples of the currently known X-ray crystal structures of pesticidal compounds in complex with their target biopolymer

Target site	Complexed ligand	PDB Code[1]
	Insecticidal	
acetylcholinesterase[2]	trifluoroacetophenone derivative	1AMN
ecdysone receptor	ponasterone A, bisacylhydrazine	1R20
juvenile hormone esterase	trifluoromethyl ketone derivative	2FJ0
nicotinic acetylcholine receptor[3]	nicotine	1UW6
	Herbicidal	
acetoxyhydroxyacid synthase	chlorsulfuron	1YHZ
	imazaquin	1Z8N
adenylosuccinate synthetase	hydantocidin 5'-monophosphate	1SOO[4]
acetyl-CoA carboxylase carboxyltransferase	diclofop	1UYR
EPSP synthase[5]	glyphosate	1G6S
fatty acid synthetase, type II	triclosan	1D7O
4-hydroxyphenylpyruvate dioxygenase	hydroxypyrazolyl ketone	1TFZ
imidazoleglycerol phosphate dehydratase	2-hydroxy-3-(1,2,4- triazolyl)-propylphosphonate	2F1D
	Fungicidal, bactericidal	
CYP450 14α-sterol demethylase	ketoconazole[6]	1JIP
	pyroquilon	1G0O
trihydroxynaphthalene reductase	azoxystrobin	1SQB
	famoxadone	1L0L
mitochondrial cytochrome *bc*1	carpropamid	2STD
	streptomycin	1NTB
scytalone dehydratase		
RNA aptamer		

[1]Protein Data Bank accession code. The X-ray structure of and additional information for the specified complex can be found at http://www.pdbj.org or http://www.rcsb.org.
[2]From the electric ray *Torpedo californica*.
[3]From the snail *Lymnaea stagnalis*.
[4]From *Escherichia coli*. The PDB code X-ray structure for uncomplexed enzyme of *A. thaliana* is 1DJ2.
[5]From *Escherichia coli*.
[6]Antifungal pharmaceutical structurally related to azole fungicides used in agriculture.

elucidation of the X-ray structure of the ecdysone receptor has paved the way for computer-aided design of new receptor agonists (Nakagawa, 2005). Based on the 3D structure of the acetylcholine-binding protein complexing nicotine, the selectivity of and resistance to imidacloprid could be interpreted (Matsuda et al., 2005).

Role of chirality in pesticide activity, toxicity and environmental biotransformation

Numerically, about a quarter of all pest control agents listed in *The Pesticide Manual* (Tomlin, 2003) are chiral compounds containing at least one asymmetric carbon atom. Often only one of the stereoisomers is responsible for the desired biological activity but, mostly for economic reasons, many of the commercialized products are mixtures of isomers as produced by chemical synthesis. It has long been recognized that the stereoisomers of a particular compound can have different toxicological properties and their biodegradation in the environment can also differ (Garrison, 2006; Hegeman and Laane, 2002). The biologically inactive isomer can merely act as a ballast. In the worst-case scenario, however, the inactive isomer degrades slowly and can affect non-target species adversely. Thus, there is an increasing need to market stereoisomerically enriched products, especially if studies indicate health or environmental risks for the unwanted, target-inactive isomer. In certain cases, proprietary reasons also stimulate the development of a chiral version of an established racemic active ingredient.

Green chemistry

The discovery of a promising pesticide has to be followed by the development of a safe manufacturing process capable of producing the material in the quantity and the highest quality required. On monetary terms, the costs associated with process development and related analytical methodologies could be commensurate with those devoted to discovery (Phillips McDougall, 2005). Because pest control agents are applied to large areas, environmental considerations such as selective toxicity and degradation studies have always been required for the registration of the active ingredient and the formulation. No such formal requirements exist for manufacturing processes, which can involve hazardous or polluting reagents and solvents. Due to health and environmental reasons, the chemical industry should to adopt the principles of green chemistry for the innovative design and production of materials to prevent and reduce pollution (Anastas and Kirchhoff, 2002; US EPA, 2006). The principles of green chemistry will increasingly be applied by the pesticide industry both for the production

of the active pesticide ingredient and for the development of environment friendly formulation types.
Actually, many of the above principles had dominated pesticide chemistry well before the early 1990s when the term green chemistry was coined. New types of selective pest control agents had been developed and adopted in pest control 10-15 years earlier. For example, originating from research carried out in the 1970s, pyrethroids and juvenoids started to replace several organophosphate and carbamate insecticides; sulfonylureas used at application rates typically below 100 g/ha appeared in weed control. In general, the application rates and non-target toxicity of pest control agents being commercialized from the 1980s are much lower than of those developed earlier. Again, advances in technology, market changes and stricter regulations drive research and development.

Combination of chemical pest control with other crop protection technologies

In 2005, the global market value of biotech crops was US$5.25 billion corresponding to about 15% of the global agrochemical sales (James, 2005). The growing adoption of crop biotechnology limits the market for chemical pesticides, especially for synthetic insecticides that are replaced by technologies based on *Bt*-producing plant varieties. The picture is less clear for the herbicide market where herbicide-tolerant traits do require the application of the given herbicide. While the global adoption of transgenic herbicide-tolerant crops existing today will certainly continue, new herbicide-resistance varieties are unlikely to be developed (Devine, 2005). This is especially true for crops for the food and feed market. However, the increasing demand for biorenewables, e.g. for plants grown for fuel or polymer production, could result in the development of genetically modified crop varieties, which might call for new, economically and environmentally viable herbicides and other pest control agents to be used in these cultivations.
Chemical means of insect control also supplement the so-called area-wide integrated pest management control strategies. For example, the mass release of sterile insects in isolated areas is typically preceded by initial population suppression by insecticides (Dyck et al., 2005).

Conclusions

The combination of traditional and automated chemical synthesis with biochemistry, metabolic engineering, and genomics offers exciting

possibilities to exploit the (bio)chemical diversity in the quest for new crop protection chemicals. Such research and development should take place in an economic era when there is no essential growth in pesticide sales. Nevertheless, the substitution of many hazardous pest control agents by safer ones will continue and new chemical and biochemical means of industrial syntheses will emerge. Rapidly advancing technologies, on one hand, and changing market demand, on the other, will continue to drive innovation in the agrochemical industry to provide customers with economically viable and safe crop protection and disease vector control agents. Over the past four decades many novel chemical prototypes have emerged. In spite of industry consolidations, the rate of introduction of new active ingredients has not changed much (Phillips and McDougall, 2003). Chemistry has played a decisive role in these developments either by finding and optimizing entirely new structural prototypes or designing analogues of natural products.

Paradoxically, the impressive advances in pesticide research do not seem to be appreciated by the general public. The pervasive "chemophobia" questions the mere necessity of the use of chemical pest control agents, especially of those of synthetic origin, in spite of the fact that these have been amongst the most thoroughly investigated, thus "transparent" substances. Considering recent advances in our understanding of the selective mode of action of many pesticides at the molecular level, and the possibilities offered by structure-based methods of compound design, saying that the time for chemicals in agriculture is over would be a mistake. The present constrains in research and discovery of new chemical pest control agents are not scientific but rather economic and political/social ones. One could agree with the recent statement by Gro Harlem Brundtland (Brundtland, 1997), currently director-general of the WHO: "Politics that disregard science and knowledge will not stand the test of time. Indeed, there is no other basis for sound political decisions than the best available scientific evidence. This is especially true in the fields of resource management and environmental protection."

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RESEARCH AND DEVELOPMENT IN PESTICIDE CHEMISTRY. CURRENT STATUS AND A GLIMPSE AT THE FUTURE

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Summary

The global agrochemical market has been around US$30,000 million for recent years. While there has been no essential growth in overall sales there have been important changes in the chemistries of all pesticide categories over the last decade. The discovery of new structural prototypes has provided new products that are able to replace some obsolete pesticides. Natural products and traditional synthetic chemistry have continued to provide valuable lead compounds for new pesticides. The biomolecular characterization of the mode of action of many pest control agents at receptor and enzyme levels renders computer-aided pesticide design in three dimensions a reality. Environmental considerations should be crucial at the end-product level as well as in manufacturing processes (green chemistry). Complementing biological and biotechnological tools, chemicals used for the production of food and fiber, for disease vector control as well as for potentially new markets (e.g., biorenewables for industrial raw materials and as energy sources) will be essential in improving the welfare of the growing world population.

THE SAFETY OF FOOD PREPARED FROM GENETICALLY MODIFIED PLANTS

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Introduction

Even a cursory look on the potential health and metabolic effects of GM-plant derived feeds/foods revels a scarcity of published data. The regulators in the USA use a decision tree approach in which they review the data provided by the biotechnology companies but do not carry out safety assessment of their own (Faust, 2002). In Europe also, the preferred approach is to use compositional comparisons between the GM crop and its traditional counterpart, and if these results show no significant differences they are considered to be "substantially equivalent", meaning that the GM is as safe as the non-GM crop. Existing legislation does not require the testing of GM crop-based feedstuffs with the target animals. Thus, the regulation of GM-plants at present is based on a rather poor legislative and scientific foundations.

The idea of substantial equivalence has outlived its usefulness. Nevertheless, it is often claimed that as there are no biologically significant compositional differences between the "substantially equivalent" GM and non-GM crops, even when they are statistically significantly different. Therefore, the definition of "substantial equivalence" is a concept without any scientific or legal definition, as it is obvious from the following statement: a BSE infected cow is substantially equivalent to a healthy one. Therefore biological tests, based on scientifically validated experimental methods and statistical analyses, which do not show any nutritional, toxicological and physiological differences, are the real requirement for determining the safety of any GMO, before one considers it to be safe as human food or animal feed. Proper biological testing of foods/feeds derived from GM-crops is made all the more urgent because without being aware of it, the majority of the population is exposed to them, since proper labeling is not being followed even in those countries, where it is required by law.

In this review we examine the only properly carried out and published human experiment, and some animal studies, which ought to have been taken into consideration before releasing any of the GM foods/feeds presently on the market, or being considered for release.

Transgene survival in the alimentary tract and its possible consequences

In genetic modification the intended gene is incorporated into the genome of a crop using a vector containing several other genes/DNA sequences, including as a minimum, viral promoters, transcription terminators, antibiotic resistance- or other marker genes and reporter genes. According to the submission of biotechnology companies to the competent authority, the transgenic DNA degrades fully in the digestive tract, therefore it cannot represent any danger to the public.

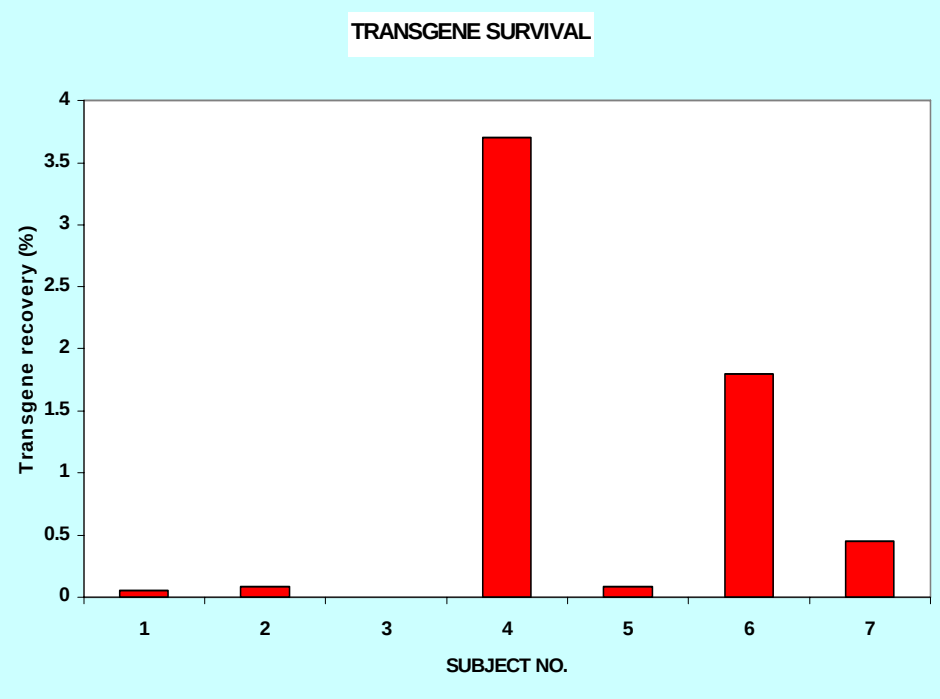
Therefore it is unfortunate that the possible physiological effects of these genes and their expressed proteins on the digestive tract and the body are seldom taken into account in spite convincing evidence that some of the transgenes included in the vector may have an effect on safety. This is particularly important, as it is now well established that DNA does not always break down in the alimentary tract (Schubbert et al., 1997; 1998; 2002). This opens up the possibility that the antibiotic resistance marker gene, in addition to others, may be taken up by bacteria in the digestive tract and contribute to the spreading of antibiotic resistance (see Experiment with Humans, below).

The observation, that a substantial proportion (6 to 25%) of a genetically engineered plasmid survived a one hour exposure to human saliva (Mercer et al., 1999) is being ignored. Partially degraded plasmid DNA also successfully transformed *Streptococcus gordonii*, which lives in the human mouth. Plasmid antibiotic resistance marker gene DNA exposed to ovine saliva could transform competent *Escherichia coli* to ampicillin resistance in vitro (Duggan et al., 2000) and when fed to chicks incorporated into GM maize the plant-derived marker was shown to be present in their crop and stomach (Chambers et al., 2002). The transfer of DNA derived from GM or non-GM plant tissues to duodenal juice, lymphocytes, internal organs, etc. of animals fed on feed rations containing these is now well established (Chowdury et al., 2003), though their physiological significance for humans is unclear.

Experiment with Humans

The only peer-reviewed human trial was carried out with GM soybean (Netherwood et al., 2004), with the aim to determine the ability of the antibiotic resistance marker gene to transfect live bacteria in the human gastrointestinal tract. This human study (Netherwood et al., 2004) confirmed the results of similar animal studies. It was shown that in the digesta of seven ileostomy patients (people whose large intestine has been surgically removed and replaced with an external pouch joined to the lower end of their small intestine), who were given a single meal (in form of a milkshake containing ROUNDUP-READY GM soybean), variable but measurable amounts of the full length transgene construct survived, and could be detected in their gut bacteria even after they were sub-cultured 4 times.

Figure 1. The survival of the full length transgene construct in the digesta of humans



Redrawn by using the original data

Even more unfortunate was the finding, that in the "0 time" (control) samples, taken before giving the patients the GM-soybean meal, the transgene was already detectable in 3 of the 7 patients. This indicates that 30-50% of the British population was already carrying the transgene in their gut bacteria, although GM-soy – in theory – was not available in Britain (most food manufacturers and supermarkets pledged not to use GM ingredients in their products).
In the same experiment with healthy individuals the transgene degradation was assayed in the feaces and, since it did not contain any detectable transgenic material, the ability of transfection was dismissed. However, this part only proves that there is no environmental contamination by undegraded transgenic DNA derived from this type of GM-soy, and provides no evidence that the transgenic DNA is not being taken up by the cells of the gut epithelium or by the gut bacteria. Therefore the prospect of the uptake of functional vector genes, including the antibiotic resistance gene, should be seriously considered and taken into account.

Experiments with Animals

The main stated objective of the GM regulation is to assure the human population that GM foods are safe while animal safety is seldom discussed. Most animal studies had limited, mainly commercial objectives, as it is obvious from recent reviews (Faust, 2002; Aumaitre et al., 2002).

GM tomatoes
Indeed, historically the first such study (still unpublished) was carried out on FLAVR-SAVR^{TM} tomato at the instigation of the FDA (Food and Drug Administration of USA). The studies, released following a court action showed necrosis/erosion of the stomach in 4, (later upon re-examination 7) out of 20 female rats fed the GM-tomato. No necrosis/erosion was found with males fed the GM-tomatoes, or with males and females fed the parent, non-GM tomatoes.
A GM tomato line developed using the *Bacillus thuringiensis* crystal protein CRYIA(b) gene may have some general relevance to GM studies. In this study (Noteborn et al, 1995) there was a commendable attempt to use immunohistology to measure the binding of the gene product to the rat gut surface *in vivo* rather than using spurious arguments why the gene product should not bind. Unfortunately, instead of the Bt toxin isolated from GM tomatoes, an *Escherichia coli* recombinant and potentially less stable form of the gene product was tested, (this is a general practice with testing all GM-derived proteins) that put a serious question mark over the results.

However, even with this recombinant form the in vitro binding of the Bt toxin to gut sections, including the caecum and colon of humans and *Rhesus* monkeys, was demonstrated by immunocytochemistry.

Herbicide-resistant soybean

In a safety study of glyphosate-resistant soybean, the feeding value, wholesomeness (Hammond et al., 1996) and possible toxicity (Harrison et al., 1996) of two GM-soy lines was compared to that of the parent line. Processed GM-soy meal-based diets were fed to rats, broiler chickens, catfish and dairy cows between four to ten weeks at the same concentrations as in commercial non-GM soybean rations. According to the authors the lines were substantially equivalent and the performance of the animals were the same on all lines. The rat study (Hammond et al., 1996) had a wider and more academic scope than the other more production-type studies. It appears that the total protein content of the diets was adjusted to 24.7 g protein/100 g diet to be iso-nitrogenous with Purina Laboratory Rat Chow by the addition of 24.8 g of GM- and parent soybean meals respectively (about 10% protein) to a base diet. All comparisons were made to rats fed commercial Purina Chow. The protein concentration in this study therefore was appreciably higher than the usual 10-16% crude protein, regarded as optimal for the rat. This extra protein could have potentially masked any possible transgene product effects. Thus, the GM-meal replaced only 8.5 and 17% respectively of the total protein of the diet. In other words, the GM protein was diluted by other dietary proteins by 12 and 6 fold, respectively, producing possible a masking effect. In spite of this, the Purina Chow-fed control male rats grew significantly better than most of the three experimental groups fed toasted soybeans (including the parental line). There were no individual data for organ weights in the paper, but the kidney weights of the raw GM-soy line-fed male rats were reported to be significantly higher than those of the controls, while the testes of the parental line-fed rats was significantly enlarged. No histology appears to have been done apart from some qualitative microscopic observations on the pancreas that has been described as showing some minimal to mild lesions.

The results of a separate studies (Teshima et al., 2000; 2002) with toasted glyphosate-resistant GM soybean in which rats and mice were fed with this GM soybean at 30% inclusion level in the diet for 15 weeks could not be seriously considered because rat growth was minimal (less than 30 g over 105 days) and mice did not grow at all on either the test or control diets.

In a paper of Malatesta et al. (2002) it was shown that the liver of mice fed on diets containing GM soybean in comparison with conventional soybean-based diets underwent significant modifications in some nuclear features. Hepatocytes in GM soybean-fed mice showed irregularly shaped nuclei, indicating high metabolic rates, increased numbers of nuclear pores,

suggestive of intense molecular trafficking and more irregular nucleoli with numerous small fibrillar centres, typical of increased metabolic rates. Nucleoplasmic and nucleolar splicing factors were also more abundant in GM-fed mice than in controls.

GM potatoes

In a mainly histology study of the ileum of mice fed with potatoes transformed with a *Bacillus thuringiensis* var. *kurstaki* CryI toxin gene and as control, the effect of the toxin itself (Fares and El-Sayed, 1998) it was shown that both the delta- endotoxin and, to a lesser extent the Bt-potato, caused villus epithelial cell hypertrophy and multinucleation, disrupted microvilli, mitochondrial degeneration and increased numbers of lysosomes and autophagic vacuoles and the activation of crypt Paneth cells. This is an important study because it showed that, in contrast to general belief, exposure of the mouse gut (ileum) to the CryI gene product has caused profound hypertrophic and hyperplastic changes in the cells of the gut absorptive epithelium and these could lead to mucosal sensitization as it was later demonstrated (Vazquez Padron et al., 1999; 2000).

The work concerning the effect on the histology of the different gut compartments of feeding rats on diets based on GM potatoes expressing the snowdrop (*Galanthus nivalis*) bulb lectin (GNA) gene (Ewen and Pusztai, 1999) revealed some major changes in gut structure and function. Young, rapidly growing rats were strictly pair-fed on iso-proteinic and iso-caloric diets supplemented with vitamins and minerals for 10 days. Histological evaluation revealead mucosal thickening of the stomach, jejunum, caecum and colon in rats fed GM-potatoes. The study showed significant differences in growth rate, organ weight and the slowing down of the humoral immune system. The most important observation was that the proliferative hyperplastic growth of the rat gut was not a GNA lectin effect but was probably either due to some other component of the gene vector used for the genetic modification and/or the disruption caused by the incorporation of the vector in the plant genome.

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THE SAFETY OF FOOD PREPARED FROM GENETICALLY MODIFIED PLANTS

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Summary

The safety evaluation of food prepared from genetically modified plants (GM-plants, plants made by recombinant DNA technology) is still based on the scientifically fraud concept of substantial equivalence. The only human trial performed up to date provided evidence that transgenic DNA material originating from Rounup-Reaady GM-soya can transfer into the bacteria resident in the gastrointestinal tract of humans. Several animal experiments showed that there are problems with feeding GM-plant containing foods. In spite of these, GM foods are considered safe by the most authorities claming that there is no evidence of harm, which of course does not mean that they are safe.

PHYTOPATHOLOGICAL SESSION

NEW VARIETY TESTING METHOD TO THE FUSARIUM HEAD BLIGHT OF WHEAT

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The Fusarium Head Blight (FHB) is one of the most dangerous diseases of wheat and relative crops in Hungary. Because of the fact that the European Community introduced a new, obligatory limit to the deoxynivalenol (DON) content (1,25 ppm) this year, its importance is getting higher and higher. The hazard of a severe FHB epidemic depends on the large proportion of cereals in the total agricultural area and the susceptible registered varieties.

According to the present practice of the National Institute for Agricultural Quality Control (OMMI), one isolate of *Fusarium culmorum* and *F. graminearum* is used, respectively for the inoculation. Seed infection is detected by the so-called filter paper-frosting method. Due to the short variety testing period of 2-3 years, and the rising significance of the disease, the OMMI is planning to introduce a new, more complex and accurate resistance testing method in the near future.

In 2006 a total of 114 winter wheat (*Triticum aestivum* and *durum*) genotypes and FHB resistant check varieties were tested in a small-plot field trial with 10 replications in Röjtökmuzsaj, Western Hungary. Inoculation was carried out on the 6th June using 2 isolates of *F. culmorum* and *F. graminearum*, respectively. Head infection was assessed 3 times, started 3 weeks after inoculation. Inoculum concentration was measured and AUDPC values were calculated. Grain yield was measured using 25 heads / plot samples with additional visual evaluation. DON content in the seed samples, as the most important component of this method, will also be detected.

It is to be expected that the new resistance testing method will provide more reliable and precise information on the resistance or susceptibility of cereals, improving the variety testing process.

THE OBSERVATION A SLIME MOULD – *BADHAMIA FOLIICOLA* – ON RAPE STEM

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The slime mould *(Badhamia foliicola)* was found on a blackleg *(Phoma lingam)* infected rape stem. The sample was collected in Röjtökmuzsaj 16. 06. 2006. This *Myxomycetes* is belonging in the order *Physarales*, where calcium carbonate is present in the peridium or in the capillitium or in both. The main mark of the family *Badhamia* is the three-dimensional capillitium formed by chalky tubes. The sporangia of *Badhamia foliicola* are sessile, purplish-grey. The peridium is poor in chalk. Spores are loosely aggregate. Up to 20 spores are in a bunch.
Badhamia foliicola is saprophyte and common on grasses. The stem of the rape was used in order to spread the spores further.
This slime mould is relatively rare in Hungary. There are six specimens in the collection of the Hungarian Natural History Museum.

Acknowledgments
I would like to express my special thanks to Holger Müller for identification *Badhamia foliicola.* Thanks for the aid of Kálmán Vánky and Ágnes Révay.

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THE ANTHRACNOSIS DISEASE OF THE TIGHT LEAF LUPIN (*LUPINUS ANGUSTIFOLIUS* L.)

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In connection with the lupin (*Lupinus* L.) species (*Lupinus albus, L. luteus*, *L. angustifolius, L. mutabilis*) which are grown in Hungary or used for plant improvement, we have experienced a previously unknown disease since 2004, namely the anthracnose. The extent of damage caused is different for each species, the biggest loss was experienced with regard to the white and yellow flowered sweet lupins that are also grown on an industrial basis. To provide a precise definition about the extent of the damage, further observations are essential.

The tight leaf or blue flowered lupin species are – as far as we are concerned – only infected by the *Colletotrichum gloeosporioides*.

To decrease the impact of this agent – on the basis of our fungicide-sensitivity surveys – captan, mankoceb, copper(I)oxid as the most suitable as well as the combinations of these with benomyl or methyl-tyophanate. Our open-air small-parcelled experiments are to be settled according to this.

Effective protection is impeded by the fact that this pesticide also affects the inside part of the seed in the case of the lupin species examined. Seed pelleting is only able to provide a partial protection, which means that stock treatments are assumed to have an important role in the future in the plant protection of this species.

In relation to heat demand, the causative fungus agent belongs to the warm demanding species. If the humidity and the temperature is high enough – considered as an ecological condition – that may nourish the fungicid infection of this lupin species, which migh be ”epidemic” as well.

IN VITRO ANTIFUNGAL ACTIVITY OF ESSENTIAL OILS ON PATHOGENS

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Applying conventional pesticides in plant protection means significant risk to the environment and the human health. For this reason the permission of several pesticides has been cancelled. Nowadays the demand of the use of plant protection products containing natural agents e.g. plant extracts is increasing. The possibility of applying essential oils against pests has got into the focus of interest in the past decade.

In the literature several data can be found about the effect of essential oils on plant pathogens. Among medicinal plants cultivated in Hungary as well, the essential oils extracted from peppermint, thyme, sweet basil, coriander, oregano, lavender, sage among others had fungistatic or fungicide effect against *Botrytis* sp., *Fusarium* spp., *Monilinia* spp. and *Sclerotinia* sp. (Arras and Picci, 1984; Shimoni et al, 1993; Caccioni and Guizzardi, 1994; Pattnaik et al, 1996; Moretti et al, 1998; Edris and Farrag, 2003; Pankaj Sharma et al, 2003; Plotto et al, 2003). However investigations were usually carried out only *in vitro* and plant protection products against pathogens have not been developed.

In Hungary very little knowledge is available about the effects of essential oils on plant pathogens. Only a few researchers deal with the investigation of this field.

Our main objective was to investigate 28 essential oils against *Botrytis cinerea*, *Fusarium oxysporum* f.sp. *cyclaminis*, *Monilinia fructigena*, *M. laxa* and *Sclerotinia scerotiorum*. As a first step *in vitro* screening was made. The effective oils will be tested *in vivo* in laboratory and small scale experiments on living plants or plant parts.

On the bases of our preliminary results the development of plant protection products containing essential oils is perspective.

[*] M. P. and G. N. equally contributed to this work
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Materials and Methods

For testing the effect of essential oils five pathogens were chosen: *Botrytis cinerea, Fusarium oxysporum* f. sp. *cyclaminis, Monilinia laxa, Monilinia fructigena,* and *Sclerotinia sclerotiorum*. During the research 28 essential oils of medicinal and aromatic plants were investigated. For comparison commercial fungicides were involved in practical dosage [*Botrytis cinerea* and *Sclerotinia sclerotiorum* –Rovral 25 FW (0.3%), *Fusarium oxysporum* f. sp. *cyclaminis* –Fundazol 50 WP (0.2%), *Monilinia* species –Chorus 75 WG (0.03%)].

Monilinia laxa and *Monilinia fructigena* were isolated from peach fruits, *Sclerotinia sclerotiorum* from sunflower stems. *Botrytis cinerea* was isolated from strawberry fruits and *Fusarium oxysporum* f. sp. *cyclaminis* from cyclamen roots. Pathogens were cultivated on Leonian malt agar (LMA) and Potato dextrose agar (PDA) at 24ºC in the dark. Pathogenicity of the isolates was confirmed by back inoculation.

The antifungal activity of the oils was compared on the basis of the inhibition of the growth of mycelia and of the germination of conidia. The inhibition of mycelial growth was tested by agar diffusion hole test and agar dilution technique using different oil concentrations. In the first case three – 11 mm diameter – holes were bored into agar plates. 100µl essential oil was placed into each hole. Petri-dishes were incubated in thermostat at 24ºC for 48 hours for the diffusion of the oils. For the agar dilution tests essential oils in different doses – 1%, 0.5%, 0.1%, 0.05%, 0.01% - were metered to hand warm PDA culture media. Then small agar disc – originated from 4-7 days old pure culture of the pathogen – was placed into the centre of agar plates. Petri-dishes were closed with parafilm, and put into thermostat. Evaluation was carried out when the pathogens overgrew the control agar plates. The growth of mycelia on treated plates was compared with that of the control.

The inhibition of the germination of conidia was examined in micro titration plates.

Oil suspensions were placed into the wells in 1%, 0.5%, 0.1% and 0.05%. To achieve better solubility Tween 20 (0.001%) was added. Suspension of conidia was obtained from the pure culture of the pathogens. Microtitration plates were incubated in thermostat at 24ºC for 24-36 hours. For the evaluation of the efficiency of the oils the ratio of the germinated conidia was counted and the length of germ tubes was measured at 100-100 conidia by microscope. Result was compared with that of the untreated, and the fungicides control.

Antifungal activity was expressed estimating the EC_{50} and EC_{90} (Effective Concentrate) values in each experiments.

Results and Discussion

Inhibition of the growth of mycelia:
During the agar diffusion hole test almost all the oils in 1% concentration caused total inhibition of the growth of the mycelia of all tested pathogens. Thus this technique was not proved to be suitable for comparative study.
In case of agar dilution method great differences could be observed among essential oils in effectiveness at the different concentrations (Figure 1).

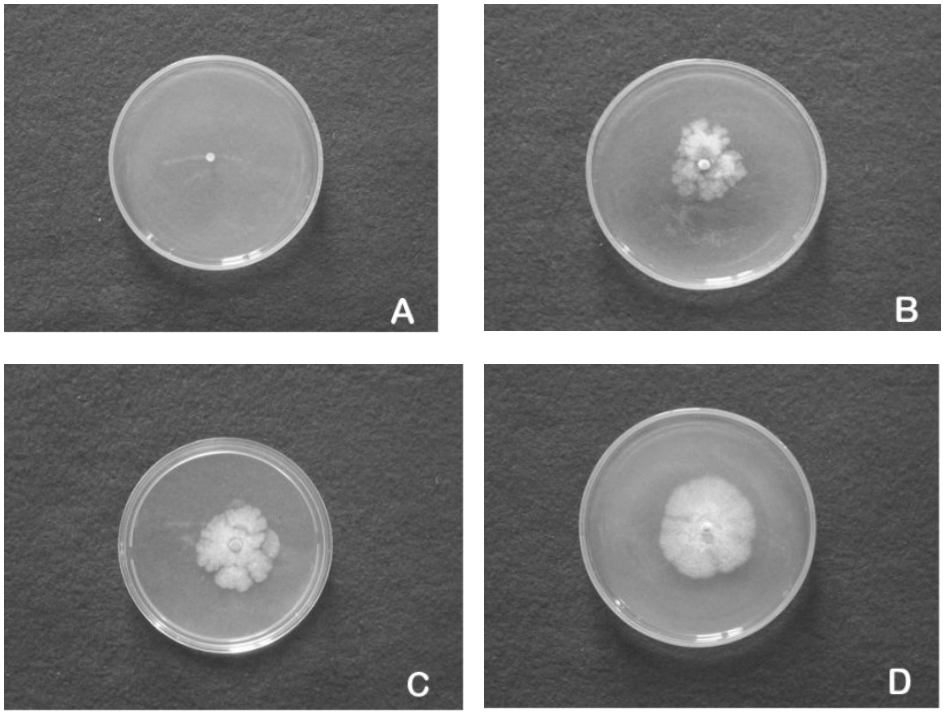

Figure 1. Mycelial growth of *Monilia fructigena* on agar plates containing 0.1% of different essential oils (A=2, B=6, C=5, D=13).

Against *Botrytis cinerea* the 16, 21 and 27 oils against *Fusarium oxysporum* f. sp. *cyclaminis* the 16, 21 and 23, against *Sclerotinia sclerotiorum* only the 27 oils gave remarkable inhibition of mycelial growth. The oils listed above resulted an EC_{50} value less than 0.01%. However none of the oils gave total inhibition in case of these pathogens. EC_{90} value ranged between 0.05 and 0.01%. In the case of *Monilinia fructigena* the growth of mycelia was absolutely inhibited even in the lowest tested concentration by the following essential oils: 2, 16, 21 and 23. (Figure 2). High inhibition level was observed also at 7, 22 and 27 essential oils. All essential oils listed above abolished the growth of mycelia of *Monilinia laxa* (Figure 3).

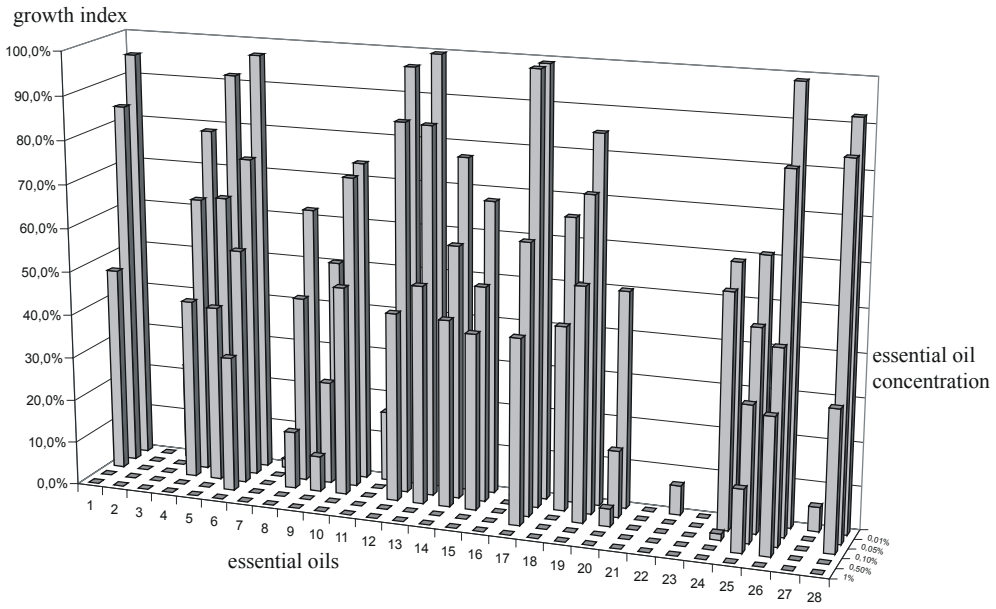

Figure 2. Effects of essential oils on mycelial growth of *Monilia fructigena*.

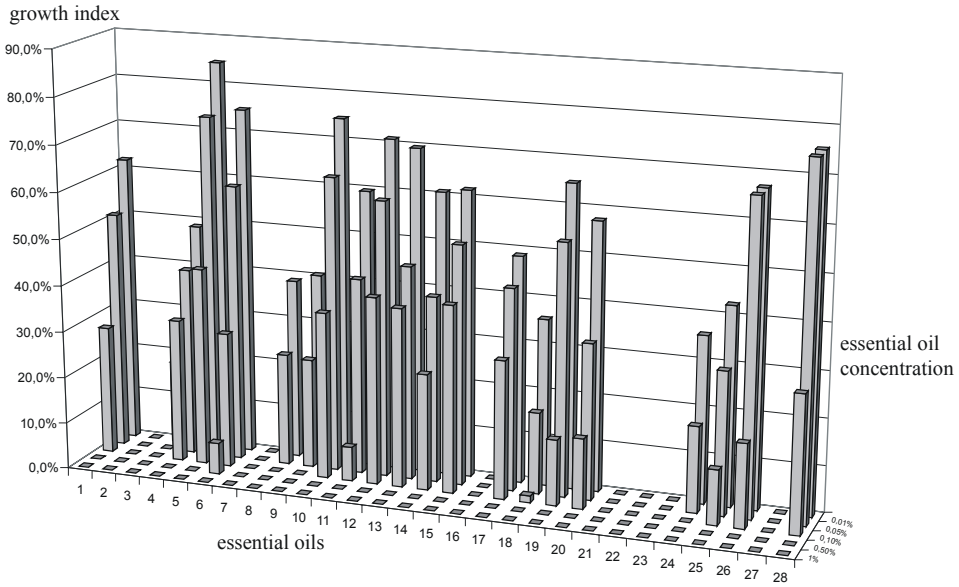

Figure 3. Effects of essential oils on mycelial growth of *Monilia laxa*.

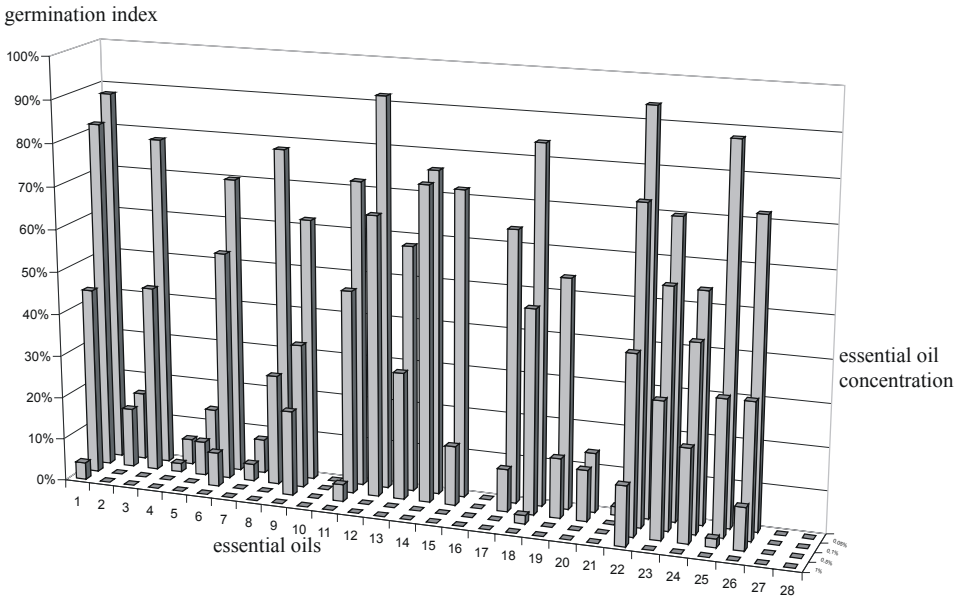

Figure 4. Effects of essential oils on conidial germination of *Monilinia laxa*

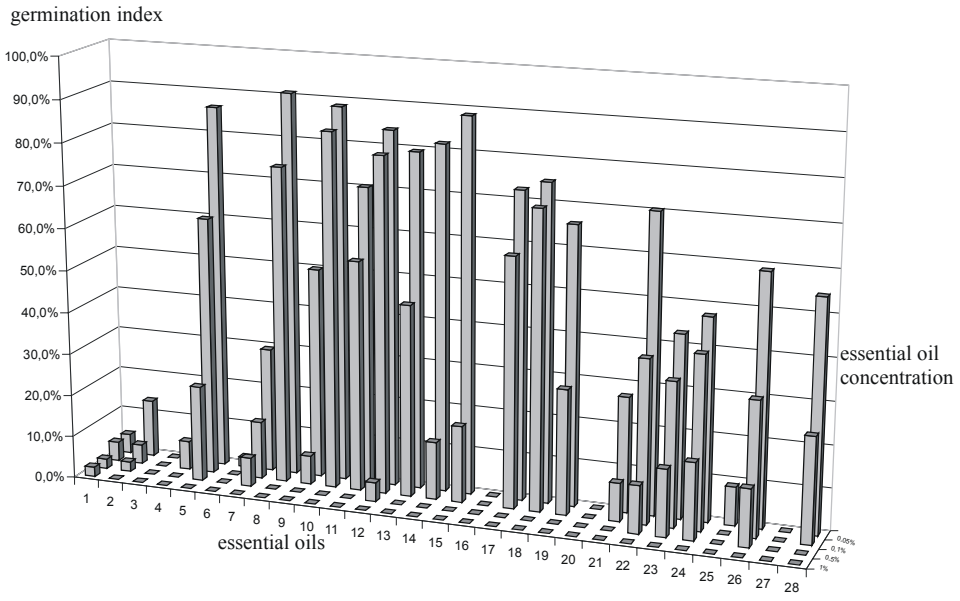

Figure 5. Effects of essential oils on conidial germination of *Monilinia fructigena*

EC_{50} value was below 0.01% at

- *Monilinia laxa*: 2, 3, 7, 9, 11, 16, 21, 22, 23 and 27.
- *Monilinia fructigena*: 2, 3, 7, 8, 9, 11, 16, 18, 21, 22, 23, 24, 25 and 27.

EC_{90} value was below 0.01% at

- *Monilinia laxa*: 2, 3, 7, 11, 16, 21, 22, 23 and 27.
- *Monilinia fructigena*: 2, 3, 7, 16, 18, 21, 22, 23 and 27.

Inhibition of conidial germination:

With the increasing of the concentration of the essential oils the number of germinated conidia of *Botrytis cinerea* in most cases proportionally decreased. In the higher concentrations the rate of conidia with short germ tubes increased. In the case of *Fusarium oxysporum* f. sp. *cyclaminis* connection between the increasing oil concentrations and the germination rate of the conidia was not consequent in many cases. Against *Botrytis cinerea* nine oils (2, 7, 10, 11, 16, 21, 22, 27, 28), against *Fusarium oxysporum* f.sp. *cyclaminis* five oils (16, 21, 24, 26, 27) resulted an EC_{50} value less than 0.05% concentration. The most effective oils were the 7, 16, 22 and 27 against *Botrytis cinerea* and the 16, 21 and 27 against *Fusarium oxysporum* f. sp. *cyclaminis*. EC90 values were less than 0.05% concentrations.

The germination of conidia of *Monilinia laxa* and *Monilinia fructigena* was inhibited by almost all tested essential oils at 1% (except 1 and 22). Remarkable differences were observed at further tested concentrations. Total inhibition of the germination of *Monilinia laxa* conidia could be noticed at 3, 16, 20 and 27 essential oils at the lowest concentration as well. The 1, 2, 4, 6 and 25 oils resulted heavy inhibition (Figure 4).

Germination of *Monilinia fructigena* conidia was completely inhibited by 10, 16, 27 and 28 essential oils and there was strong inhibition at the 4, 7 and 21 oils (Figure 5).

EC_{50} value was below 0.05% at

- *Monilinia laxa*: 1, 2, 3, 4, 6, 7, 16, 20, 21, 25 and 27.
- *Monilinia fructigena*: 2, 4, 5, 7, 8, 10, 16, 20, 21, 27 and 28.

EC_{90} value was below 0.05% at

- *Monilinia laxa*: 1, 3, 4, 6, 16, 20, 25 and 27.
- *Monilinia fructigena*: 4, 7, 10, 16, 21, 27 and 28.

The obtained results by the two experimental methods were similar. In the case of *Botrytis cinerea*, *Fusarium oxysporum* f. sp. *cyclaminis* and *Sclerotinia sclerotiorum* the 16, 21 and 27 essential oils inhibited the most the growth of mycelia and the germination of conidia.

The mycelial growth of *Monilinia* species was effectively inhibited by 2, 3, 7, 16, 21, 22, 23 and 27 oils. The germination of conidia was suppressed by 4, 16 and 27 oils in the highest extent.
On the bases of the results these oils are selected for further *in vivo* experiments.

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IN VITRO ANTIFUNGAL ACTIVITY OF ESSENTIAL OILS ON PATHOGENS

***Marietta Petróczy[1] –*Géza Nagy[1]–Rudolf Bánátfy[2] –László Palkovics[1]**

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Summary

Twentyfour essential oils extracted from mediterranean, tropical and continental plant species were tested for their effectiveness against 5 pathogens: *Botrytis cinerea, Fusarium oxysporum* f. sp. *cyclaminis, Monilia fructigena, M. laxa* and *Sclerotinia sclerotiorum.* Conventional fungicides were also involved as controls.

The antifungal activity of the oils was compared on the basis of the inhibition of the growth of mycelia and of the germination of conidia. The inhibition of mycelial growth was tested by agar diffusion hole test and agar dilution technique using different oil concentrations. The inhibition of the germination of conidia was examined in micro titration plates. Antifungal activity was expressed estimating the EC_{50} and EC_{90} (Effective Concentrate) values. In reference all the 5 tested pathogens an excellent *in vitro* antifungal activity could be observed in case of essential oils numbered 16, 21 and 27.

DOMINANCE OF THE BARLEY YELLOW DWARF VIRUSES IN WINTER BARLEY BREEDING MATERIALS OF KOMPOLT[‡]

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Barley yellow dwarf viruses (BYDVs) are the most widely distributed and the most economically important virus pathogens of cereal crops. BYDVs are not mechanically transmissible, nor through the seeds, but are transmitted by aphids as a persistent, circulative but non-propagative manner. Aphids acquire and transmit BYDVs while feeding on the phloem sieve tube elements of host plants.

BYDVs are restricted to the Poaceae (Gramineae). Cultivated hosts include all the major cereal crops: barley, maize, oat, rice, rye and wheat, as well as many annual and perennial cultivated and wild grasses. Symptoms caused by BYDVs differ with the host species and cultivar, the age and the physiological condition of the host plant at the time of infection, the strain and the environmental conditions; and they can be easily confused with nutritional and abiotic disorders.

In barley, the first symptoms are a diffuse or blotchy yellowing near the leaf tip, bright yellow discoloration then extends towards the leaf base leaving a strip of green along the side of the midrib. Plants are usually stunted, with a decrease in tiller number and biomass and a weak root system. Suppressed heading, sterility and failure to fill grains occur in the most severe cases. In the field, symptoms usually appear as yellow patches of stunted plants. Plants infected at the seedling stage suffer the most severe disease and may die without flowering. Generally, the older the plant at the time of infection less damaged by the disease. Susceptibility of plants and disease severity on the great variety of cereal cultivars available today differ considerably.

Several strains or serotypes of BYDV have been differentiated on the basis of vector specificity, the efficiency of transmission by aphids, serological and molecular biological properties.

[‡] This work was supported by the National Committee for Technological Development (OMFB) and the Agency for Research Fund Manegement and Research Exploitation (KPI).

Rochow (1969) differentiated four strains of BYDV by their relative vector specificity in transmission to the oat, variety Coast Black and in virulence on the host plant.
These strains were named relating to their predominant aphid vectors. One strain (RPV) was specifically transmitted by *Rhopalosiphum padi*, the second strain (MAV) specifically by *Macrosiphum avenae* and the third strain (RMV) specifically by *Rhopalosiphum maidis.* The fourth strain (PAV) was transmitted non-specifically by both *Rhopalosiphum padi* and *Macrosiphum avenae.*
Two strains (RPV and RMV) were found to be weakly virulent, the MAV strain was moderately virulent and the PAV strain was strongly virulent on the oat variety Coast Black.
Gill (1969) described a fifth strain of BYDV in Manitoba, which was specifically transmitted by *Schizaphis graminum.* This vector-specific strain was weakly virulent on the oat variety Clintland 64.
The five known BYDV strains were differentiated based on the apparent specificity of aphid transmission of each strain.
Lister and Rochow (1979) reported that the development of the enzyme-linked immunosorbent assay (ELISA) for BYDV eliminated the need for the aphid transmission test for the detection of BYDV strains. Because of their specificity, these assays quickly became a valuable tool for comparing and classifying BYDV strains.
Rochow (1979,1982) compared the results of the aphid transmission test with those obtained using ELISA. In comparison with the aphid transmission test, the ELISA was more sensitive and took less time. The ELISA was especially simplier for the diagnosis of mixed infection with the BYDV strains.
There are many reports concerning the occurrence and strain dominance of BYDV in cereals around the world. In North America, a cereal disease characterised by yellowing, stunting and decreased yields was observed, sometimes in epidemic proportions.
Widespread outbreaks with significant yield losses that were probably caused by BYDV occurred in 1907 and 1949. Therefore, research on the epidemiology of BYDV was begun on this continent.
There are many reports concerning the strain incidence and dominance of BYDV strains in different cereal species in the United States.
Gildow et al.(1987) found that 82 % of the 300 plants testing positive for BYDV contained the PAV strain alone or in mixed infection, compared with 19 % for RPV, 9 % for MAV and 4 % for RMV strains. No SGV strains were identified in Pennsylvania during 1984-1986.
In Illinois, Azzam and D'Argy (1989) reported that PAV was the prevailing strain, with the RPV and MAV strains occurring at low frequency.

In Idaho, Foster et al.(1990) reported that the SGV strain appeared to play a significant role in BYDV epidemiology during 1977 and 1985.
Hewings and Eastman (1995) reported that surveys on the incidence of BYDV strains throughout North America suggested that PAV was the dominant strain in most areas but that the incidence of the strain alone and in mixed infections differed from region to region and year to year. In areas where the PAV strain usually dominated, other strains occurred occasionally in epidemic proportions.
It sould be mentioned that BYDV was first identified in Europe, in the Netherlands by Oswald (1951), and was confirmed in the U.K. (Watson and Mulligan, 1957).
Plumb (1977) and Holmes (1985) also stated that the PAV, RPV and MAV strains could be found very often in perennial grasses. The strains are subsequently transmitted to cereals are often determined by the prevalence and feeding preference of the main aphid vectors rather than the availability of virus strains.
The first report on the occurrence of BYDV in winter barley in Hungary was made more than 40 years ago by Szirmai (1967). It was based on visual observations and confirmed by aphid transmission tests. In 1982 a very severe epidemic occurred in the Hungarian barley growing areas, with yield losses caused by BYDV ranging from 27 to 100 % in the different barley varieties (Pocsai and Kobza,1983).
Systematic work on the frequency of BYDV strains in cereals in Hungary has been in progress since 1994.
Pocsai et al. (1995) reported that all the five BYDV strains were present in Hungary. They demonstrated that, among the BYDV strains, the PAV strain was dominant in cereals. In maize both RPV and RMV were present at high rates.
Pocsai et al. (1996, 1997) found that BYDV-RMV was the dominant virus identified in breeding materials of winter barley at Kompolt both in 1995 and 1996.
Pocsai et al.(2001) tested the yearly variation of the dominance BYDVs at different locations of Hungary. Between 1996 and 2000 the virus dominance in winter barley changed from year to year at Kompolt. In 1996 and 1997 BYDV-RMV was the most prevalent. In 1998 the BYDV-PAV occurred at the highest rate. In 1999 the BYDV-MAV was present at the highest rate, while BYDV-MAV was dominant virus in 2000.
The BYDV strains have been separated into two major subgroups based on serological relationships, cytological effects and genome organisation. The first subgroup includes the BYDV-MAV, BYDV-PAV and BYDV-SGV strains. The second subgroup includes the BYDV-RPV, BYDV-RMV and BYDV-RGV strains.

The taxonomy of BYDV strains has been modified several times since the first classification based on vector specificity (Rochow, 1969). This classification has proved very useful but the aphids on which this classification was based were not endemic worldwide, thus in some areas other aphids and strains occurred. It has become obvious that more than one characteristic should be used to classify BYDV strains.

Pringle (1998) summarized the new taxonomic proposals approved by the Executive Committee of the International Committee on the Taxonomy of Viruses which included proposals for the family of Luteoviridae.

Fauquet and Mayo (1999) gave a list of virus names and their abbreviations and assigned the family and genus to which the given virus belonged.

According to this list, BYDVs consist of five viruses, BYDV-GPV, BYDV-MAV, BYDV-PAV, BYDV-RMV and BYDV- SGV, belonging to the family Luteoviridae. Among these, BYDV-MAV and BYDV-PAV belong to the genus Luteovirus. The remaining three viruses were classified as unassigned within the family Luteoviridae. The name of the BYDV-RPV strain was changed to *Cereal yellow dwarf virus (*CYDV-RPV) which belongs to the genus Polerovirus within the family Luteoviridae.

In many countries where BYDV has been studied, efforts have focused on reducing yield losses, and very little is known about the incidence and dominance of BYDV strains or the role of particular aphid vector species. Lack of this information may lead to false conclusions in some geographic regions.

The aim of our study was to determine the dominance of BYDVs in barley breeding materials of Kompolt.

Materials and Methods

In 2006, the incidence of Barley yellow dwarf viruses (BYDV-MAV, BYDV-PAV, BYDV-RMV, BYDV-SGV) was studied in barley breeding materials of Kompolt. Surveys were carried out in breeding lines showing leaf yellowing and stunting symptoms. Altogether 490 samples were collected for virus testing, viz. for determination of virus dominance. The leaf samples collected were homogenized using a leaf pressing machine with the addition of ELISA sample buffer solution at a ratio of 1:10. Virus diagnosis was carried out using DAS-ELISA for the detection of Barley yellow dwarf viruses (BYDV-MAV, BYDV-PAV, BYDV-SGV) from leaf samples exhibiting symptoms. The diagnostic materials used for Barley yellow dwarf viruses viruses (BYDV-MAV, BYDV-PAV, BYDV-SGV) were made by Agdia and (BYDV-RMV) was a DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) product. The serological reactions were evaluated using a Labsystems Multiscan Plus photometer at 405 nm.

Results

Incidence of BYDV-MAV, BYDV-PAV, BYDV-RMV and BYDV-SGV in breeding lines of winter barley collected in 2006 at Kompolt are illustrated in Table 1.

According to the results of ELISA test, 337 plants of the 490 plants of winter barley tested were found to be infected with BYDVs. It was found that 66.7 % of the 337 plants testing positive for BYDVs contained the BYDV-MAV alone or in mixed infection, compared with 24 % for BYDV-PAV, 18.6 % for BYDV-RMV and 19.8 % for BYDV-SGV.

As the data show, BYDV-MAV occurred at the highest ratio in breeding materials of winter barley.

Only 2 of the 49 breeding lines tested were free from BYDVs. These breeding lines were K-99/16-5 and K-98/4-30. The infection degree of BYDVs varied from 10 % to30 % in five breeding materials (K-00/10-13, Plaisant,KH-03-24-6,K-98/16-28 and K-97/16-28), showing they had high level of resistance against BYDVs. The majority of the breeding materials proved to be very susceptable to BYDVs.

A contrasting tendency can be seen between the incidence rates of BYDV-MAV and BYDV-PAV in breeding lines of winter barley. With a rise of incidence in the BYDV-MAV, the proportion of BYDV-PAV decreased, and vice-versa.

The virus dominance of BYDV is complex and influenced by many biotic and abiotic factors. The interactions between dominant viruses and vectors has a significant impact on virus epidemiology.

Several authors have reported some degree of interference competition between BYDV-MAV and BYDV-PAV in infected post plants (Smith 1963, Jedlinski and Brown 1965, Aapola and Rochow 1971, Halstead and Gill 1971, Wen et al. 1991). If a host plant acquires BYDV-MAV and BYDV-PAV at the sama time, BYDV-PAV interferes with BYDV-MAV replication, leading to lower BYDV-MAV concentration in the phloem. Because of this lower concentration, there is a lower probability of BYVV-MAV being acquired and transmitted by aphids (Gray et al. 1991)

In direct competition within the host plant, BYDV-PAV is the stronger competitor.

Viruses also compete within aphid vectors. When both BYDV-MAV and BYDV-PAV are present within aphids, BYDV-MAV inhibits the transmission of BYDV-PAV. Within vectors, BYDV-MAV appears to be the more effective competitor.

Table 1. Results of ELISA tests of the breeding lines of winter barley selected from the virus nursery at Kompolt

Plot numbers	Breeding lines of winter barley	Numbers of samples tested	Results of virological tests			
			BYDV-MAV	BYDV-PAV	BYDV-RMV	BYDV-SGV
1	K-01/80-2	10	1	5	7	0
2	K-01/80-3	10	7	2	0	0
6	KH-03-24-6	10	8	2	6	0
10	K-00/178-9	10	9	0	2	0
15	Plaisant	10	2	1	0	1
16	Botond	10	9	2	2	0
20	K-00/10-10	10	3	5	1	1
26	K-96/80	10	8	9	0	1
28	K-00/186-2	10	0	4	0	1
36	Murcie	10	9	7	6	0
37	K-00/10-13	10	0	1	0	0
47	K-93/154-4/4	10	7	7	0	1
59	K-99/16-5	10	0	0	0	0
63	K-00/189-2	10	0	4	0	2
65	K-92/141-16-/3-3	10	0	5	0	1
78	K-99/51	10	0	6	0	2
79	KH-03-24-6	10	0	3	0	2
83	K-92/173-3/2-4	10	0	4	0	3
89	AMÁ-1	10	10	2	3	0
98	K-97/16-2	10	1	5	3	0
106	GK-Puszta	10	8	7	3	5
109	K-00/22-2	10	8	4	2	4
115	K-01/154-3	10	0	5	1	0
122	K-94/166-117	10	10	5	0	1
133	K-01/179-2	10	10	2	3	0
135	K-99/109-3	10	5	5	0	1
139	K-94/166-11/2	10	6	7	0	0
144	Viktor	10	4	6	5	8
148	K-97/16-21	10	7	1	5	0
150	K-98/4-29	10	9	9	6	5
151	K-00/25	10	0	3	0	1
165	K-97/16-22	10	0	3	0	5
166	K-97/16-23	10	10	0	0	0
167	K-98/4-30	10	0	0	0	0
172	K-98/149-12	10	9	4	0	1
183	K-97/16-24	10	6	1	1	0
189	K-98/149-13	10	0	1	0	0
200	K-00/27-2	10	0	6	0	0
202	K-97/16-28	10	1	1	0	0
217	K-00/28-1	10	10	3	2	3
220	K-97/16-30	10	6	3	3	5

Table 1. (continued)

223	K-00/134-8	10	9	4	0	0
231	Catánia	10	6	3	1	2
232	KH-Agria	10	7	2	0	2
238	K-97/16-33	10	5	3	0	4
250	Eszter	10	2	4	0	0
256	K-97/16-39	10	9	4	0	4
277	K-00/150-1	10	7	1	1	0
295	K-00/153-1	10	7	0	0	1
		490	225	81	63	67

The relation between aphid vectors and infected host plants may heve also influenced the decline of BYDV-PAV and the dominance of BYDV-MAV. Host plant infection results physiological changes in host plants, including changes in nitrogen metabolism, and aphids respond strongly to plant nitrogen status. So, aphid population dynamics, host choice behavior could all be affected by host plant infection.

The explanation for such changes involves a complex interaction between the different vectors, the host plant for both vector and virus, and the weather conditions. This study on the dominance of BYDVs in breeding materials was aimed at a better understanding of the epidemiology of cereal viruses.

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DOMINANCE OF THE BARLEY YELLOW DWARF VIRUSES IN WINTER BARLEY BREEDING MATERIALS OF KOMPOLT

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Summary

In 2006, the incidence of Barley yellow dwarf viruses (BYDV-MAV,BYDV-PAV, BYDV-RMV, BYDV-SGV) were studied in breeding materials of winter barley of Kompolt for the determination of virus dominance. Surveys were carried out in breeding lines sown in virus nursey showing leaf yellowing and stunting symptoms at Kompolt
Altogether 490 samples were collected for virus testing. Virus diagnosis was carried out using DAS-ELISA for the detection of Barley yellow dwarf viruses (BYDV-MAV, BYDV-PAV, BYDV-SGV) from leaf samples exhibiting symptoms. The diagnostic materials were made by Agdia and (BYDV-RMV), a DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH), respectively. The serological reactions were evaluated using a Labsystems Multiskan Plus photometer at 405 nm.
According to the results of ELISA test, 337 plants of the 490 plants of winter barley tested were found to be infected with BYDVs. It was found that 66.7 % of the 337 plants testing positive for BYDVs contained the BYDV-MAV alone or in mixed infection, compared with 24 % for BYDV-PAV, 18.6 % for BYDV-RMV and 19.8 % for BYDV-SGV. As the data show, BYDV-MAV was the dominant virus in breeding materials of winter barley of Kompolt.This study on the dominance of BYDVs in breeding materials was aimed at a better understanding of the epidemiology of cereal viruses.

ANALYSIS OF SOME RECOMBINANT *PLUM POX VIRUS* (PPV) ISOLATES FROM BULGARIA, THE COUNTRY WHERE PPV WAS FIRST RECORDED

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Plum pox virus (PPV), the casual agent of the Sharka disease, is the most important viral pathogen of *Prunus* trees and poses a serious threat to stone fruit plantations (Németh, 1986). The disease was first described in Bulgaria (Atanasoff, 1932), since than PPV has spread throughout Europe (Roy and Smith, 1994) and nowadays it presents all over the world except Australia (Wetzel et al., 1991; Acuña, 1993; Thakur et al., 1994; Milius, 1999; Damsteegt et al., 2001; Thomson et al., 2001).
PPV is a member of the *Potyvirus* genus within the *Potyviridae* family. It has a single-stranded positive-sense genomic RNA of about 10,000 nucleotides with a single open reading frame encoding a polyprotein from which 10 functional proteins are released (Riechmann et al., 1992).
Analysis of a large number of PPV isolates has permitted the identification of three major (PPV-M, PPV-D, PPV-Rec) and two minor (PPV-EA, PPV-C) PPV subgroups to date (Nemchinov et al., 1996; Wetzel et al., 1991; Candresse et al., 1994; Glasa et al., 2004; Szemes et al., 2001). An unusual PPV isolate reported from Canada (James et al., 2003) may present a third distinct minor subgroup (PPV-W).
PPV-Rec subgroup corresponds to an ensemble of closely related isolates characterized by a homologous ancestral recombination event between PPV-M and PPV-D with a recombination break point located in the 3' part of the NIb gene, similar to that initially reported for the PPV-o6 isolate (Cervera et al., 1993).
In order to achieve data about PPV subgroups of Troyan region, Bulgaria, samples were taken from naturally infected plum (*Prunus domestica* L.) trees.

Materials and Methods

PPV isolates were collected from different plum varieties showing the same type of symptoms in the experimental field of the Research Institute of Mountain Stockbreeding and Agriculture, Troyan, Bulgaria in 2004. The

isolates were sap-transmitted to *Nicotiana clevelandii* and *N. benthamiana* indicator plants and investigated in this study.

Total nucleic acid (TNA) was extracted from two small leaf discs of infected *N. benthamiana* test plants by the method of White and Kaper (1989). cDNA was synthesized by reverse transcription (RT) of TNA using M-MuLV reverse transcriptase and universal primer $PolyT_2$. PCR amplification was performed using *Taq* DNA polymerase and a Potyvirus specific oligonucleotid primer-pair Poty7941 [5'-GGAATTCCCGCGG (AGCT)AA(CT)AA(CT)AG(CT)GG(AGCT)CA(AG)CC-3', sense primer, contained a *Sac*II restriction site (marked in bold)] and PolyT [5'-CGGGG ATCCTCGAGAAGCTTTTTTTTTTTTTTT-3', antisense primer (Deborré et al., 1995), contained a *Bam*HI site (marked in bold)] to amplify the 3' end of the nuclear inclusion b (NIb) gene, the whole coat protein (CP) gene and the 3' untranslated region (UTR) starts at the polyA tail of the virus. The following conditions were used for the PCR: initial denaturation at 94 ºC for 3 minutes, 40 cycles of 94 ºC for 15 s, 50 ºC for 30 s and 72 ºC for 3 min and a final extension at 72 ºC for 10 min. The purified PCR products were digested with *Sac*II and *Bam*HI restriction endonucleases and the resulting fragments were inserted into plasmid pBluescript SK^+ (Stratagene) digested with the same enzymes and were transformed into *Escherichia coli* DH5α competent cells. The recombinant plasmids were sequenced using by an automated DNA sequencer (Applied Biosystem Gene Analyzer 3100). The partial genomic sequences were finally assembled from the overlapping sequences of the cDNAs and sequence analysis was carried out. Sequence comparisons were performed using the GAP program of the Wisconsin Package Version 10.0 Genetic Computer Group (GCG), Madison, Wisc. sequence analysis software. The alignment achieved by GAP program was used as input data to construct phylogenetic tree with the neighbour-joining distance method implemented in CLUSTAL W. The tree was visualized using the program DRAWTREE.

Results and discussion

Six PPV isolates collected from different plum varieties showed typical symptoms of PPV infection: chlorotic spots and/or rings on the leaves. Five isolates out of six could be sap transmitted to test plants and were investigated in this study (see Table 1.).

Table 1. PPV subgroup of isolates investigated in this study and accession numbers of the partial sequences determined.

Isolate	Origin	Subgroup	GenBank accession numbers
PPV-Troy1	*P. domestica* cv. Chachanska rodna	PPV-Rec	AM260933
PPV-Troy2	*P. domestica* cv. Stanley	PPV-D	AM260934
PPV-Troy4	*P. domestica* cv. Kjustendilska	PPV-Rec	AM260935
PPV-Troy5	*P. domestica* cv. Gabrovska	PPV-Rec	AM260936
PPV-Troy6	*P. domestica* cv. Hanita	PPV-Rec	AM260937

The (Cter)NIb–CP–3'UTR region of all isolates were amplified by RT-PCR. The PCR products were cloned and sequenced.
The coat proteins (CPs) of all isolates were identical in size (330 aa residues long) and the DAG motif associated with aphid transmission (Atreya et al., 1995) was found in all sequences.
Amino acid sequence comparisons were done among each Bulgarian isolate. The highest level of similarity was found between Troy4–Troy6 and Troy4–Troy5. Significant homology was found also between Troy4–Troy1 and Troy5–Troy6 isolates, while the lowest percent of similarity was found between Troy2 and the other four isolates (see Table 2.).

Table 2. Percentage of nucleic acid sequence homology (above the diagonal) and amino acid sequence homology (below the diagonal) of CP of some PPV isolates.

	PA	Ran	SK68	PS	Pd4	BOR-3	Troy1	Troy2	Troy4	Troy5	Troy6	SoC	W	El-Amar
PA		98.79	87.17	86.97	86.67	86.77	85.76	99.60	86.47	86.26	86.97	80.12	78.01	80.61
Ran	98.18		87.58	87.37	87.07	87.17	86.16	98.79	86.87	86.67	87.37	80.33	78.62	80.61
SK68	91.82	92.73		98.28	96.87	96.77	95.68	87.27	96.36	96.36	96.77	80.65	78.62	82.32
PS	92.12	93.03	99.39		96.36	96.26	95.25	87.07	95.96	95.96	96.36	80.04	78.42	82.02
Pd4	92.12	93.03	97.88	97.88		99.49	98.18	86.97	98.99	98.99	98.89	80.14	78.22	82.32
BOR-3	91.82	92.73	97.88	97.88	99.09		98.08	87.07	98.89	98.89	98.99	80.24	78.42	82.53
Troy1	89.70	90.60	95.76	95.76	96.97	97.27		86.06	97.98	97.98	97.68	79.53	77.81	81.41
Troy2	99.09	98.49	92.42	92.73	92.73	92.42	90.30		86.77	86.57	87.27	80.43	78.12	80.81
Troy4	91.52	92.42	97.58	97.58	98.79	99.09	97.58	92.12		99.19	98.49	79.74	78.12	82.02
Troy5	90.60	91.52	96.67	96.67	97.88	98.18	96.67	91.21	98.49		98.49	79.74	78.01	82.02
Troy6	92.12	93.03	97.58	97.58	98.79	99.09	96.97	92.73	98.79	97.88		80.45	78.32	80.63
SoC	85.02	85.63	87.46	87.77	87.16	87.16	84.50	85.63	87.16	87.08	87.46		80.97	76.03
W	85.93	85.37	86.89	86.59	85.71	85.71	85.67	86.85	85.71	84.80	85.71	83.69		79.05
El-Amar	86.02	86.63	87.27	87.88	87.27	87.88	86.36	86.63	87.58	86.67	86.97	82.48	85.76	

Most of the differences in the CPs of these five isolates were located in the amino terminal part of the CP. The core and C-terminal regions of all isolates were highly homologous.
The amino acid sequences of these Bulgarian PPV isolates were compared with amino acid sequences of other characterized PPV sequences available

from the GenBank database (http://www.ncbi.nlm.nih.gov) presented in Table 2. Four isolates, Troy1, Troy4, Troy5 and Troy6 were most similar to BOR-3 (Glasa et al., 2004) (a representative member of PPV-Rec subgroup), while Troy2 was almost identical to PA isolate (a representative member of PPV-D subgroup). This result is in good agreement with the phylogenetic analysis performed using the nucleotide sequence data of the (Cter)NIb–CP regions. The phylogenetic tree clearly showed the clustering of Troy1, Troy4, Troy5 and Troy6 isolates with other previously characterized recombinant isolates, while Troy2 clustered with PPV-D isolates (see Figure 1).

The nucleotide sequences reported in this work have been deposited in the GenBank database and assigned the accession numbers: AM260933, AM260934, AM260935, AM260936, AM260937.

The accurate identification of isolate subgroups is among the first steps in the development of effective disease-control strategies. Formerly the vast majority of the identified PPV isolates was assigned to either one of two major subgroups, PPV-M and PPV-D (Bousalem et al., 1994). Recently a number of recombinant PPV isolates have been detected in several European countries and therefore a third major subgroup, PPV-Rec has been determined (Glasa et al., 2004).

In this study, analysis of a limited number of Bulgarian PPV isolates showed that with the exception of the Troy2 identified as belonging to PPV-D, all other isolates could be classified as belonging to the PPV-Rec subgroup on the basis of amino acid sequence homologies and the presence of conserved amino acids in the CPs. Despite the fact that a relatively small number of isolates were tested this is in good agreement with the results of Kamenova et al. (2001) that PPV-D isolates are relatively rare (around 5 %) in Bulgaria, while be in contrast with the observation that PPV-M is the dominant strain (incidence of 88 %), however PPV-Rec subgroup is a special branch in PPV-M subgroup. The CP of PPV-Rec isolates corresponds to PPV-M due to the recombination point is upstream of the CP in the NIb gene (Glasa et al., 2004).

It is mentionable that out of the five amino acids (K_4, I_{38}, T_{42}, I_{58}, T_{68}) that are specifically conserved in all recombinant sequences characterized to date – except Pd4 isolate, which has A_{68} – in the 3' end of the CP (Glasa et al., 2004; Salamon and Palkovics, 2002) all could be found in Troy1, Troy4 and Troy5, while in the CP of Troy6 there was valine instead of isoleucine at amino acid position 38. On the other hand our sequence data support the observation that differences between the amino acid sequences of isolates belong to different major PPV subgroups (PPV-M, PPV-D, PPV-Rec) located mainly in the N-terminal part of the CP and there are two amino

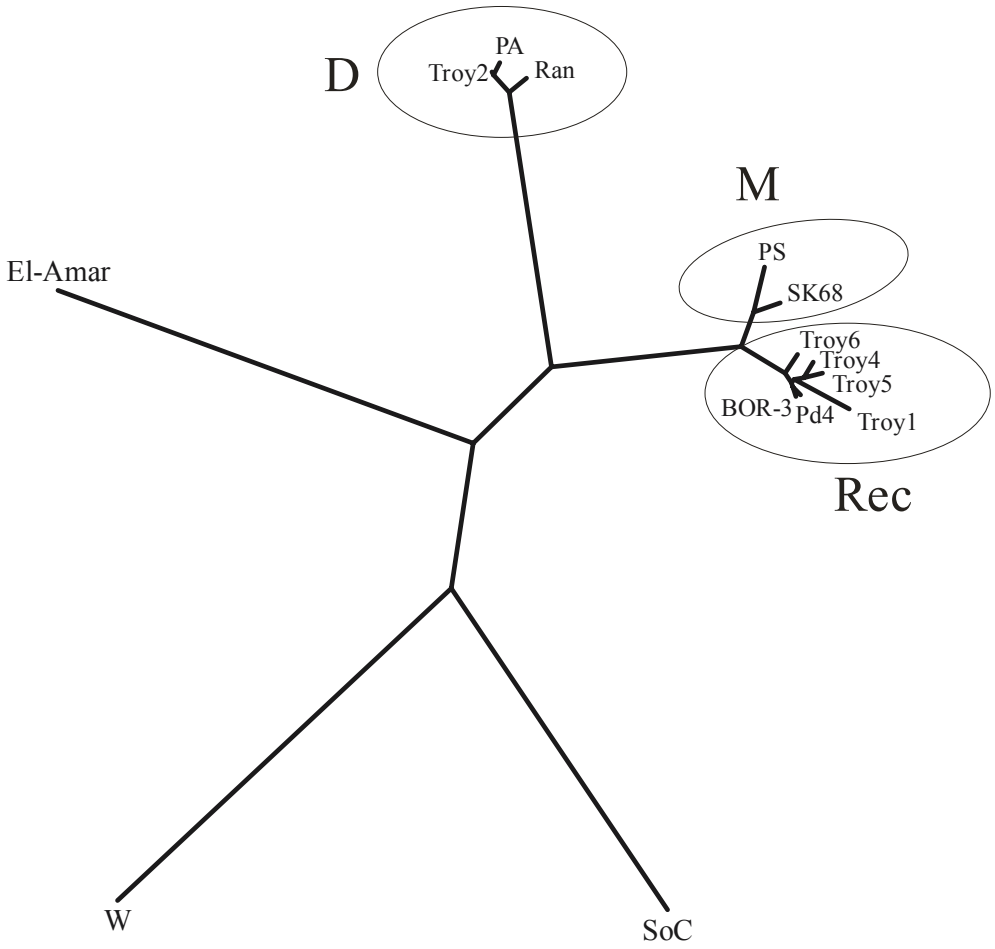

Figure 1. Phylogenetic tree of PPV isolates derived using the Wisconsin Package Version 10.0 Genetic Computer Group (GCG), Madison, Wisc. from the nucleotide sequence of coat protein gene. The following isolates were analysed: PA (GenBank accession no. AJ000340), Ran (GenBank accession no. M21847), SK 68 (GenBank accession no. M92280), PS (GenBank accession no. AJ243957), Pd4 (GenBank accession no. AJ566344), BOR-3 (GenBank accession no. AY028309), SoC (GenBank accession no. AY184478), W (GenBank accession no. AY912055), El-Amar (GenBank accession no. AY847269) and Bulgarian isolates: Troy1 (GenBank accession no. AM260933), Troy2 (GenBank accession no. AM260934), Troy4 (GenBank accession no. AM260935), Troy5 (GenBank accession no. AM260936), Troy6 (GenBank accession no. AM260937)

acid positions (58 and 68) that are able to differentiate between all three major subgroups (Salamon and Palkovics, 2002).

Acknowledgements

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ANALYSIS OF SOME RECOMBINANT *PLUM POX VIRUS* (PPV) ISOLATES FROM BULGARIA, THE COUNTRY WHERE PPV WAS FIRST RECORDED

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Summary

Troyan region is an important plum growing area in Bulgaria, the country where *Plum pox virus* (PPV) was recorded for the first time. In an effort to characterize PPV strains of this region six samples were collected from naturally infected plum (*Prunus domestica* L.) varieties in 2004 in the experimental field of the Research Institute of Mountain Stockbreeding and Agriculture in Troyan (Bulgaria).

PPV subgroup typing was performed using RT-PCR followed by sequence analysis of the (Cter)NIb–CP–3'UTR region of each Bulgarian PPV isolate. Amino acid sequence comparisons of the RT-PCR amplified part of the virus genome revealed that four isolates out of the five belonged to the PPV-Rec subgroup. These recombinant isolates were almost identical at the molecular level and shared the same recombination breakpoint in the NIb gene as well as a typical signature in their amino-terminal CP sequence. While only one isolate could be classified as a member of PPV-D subgroup.

RESULTS OF THE USE OF *CRYPHONECTRIA PARASITICA* HYPOVIRULENT STRAINS IN HUNGARY AND IN SLOVAKIA

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Cryphonectria parasitica (Murrill) Barr, the causal agent of the chestnut blight disease was introduced into Europe around 1938 (Biraghi 1946) and led to considerable destruction of the European chestnut (*Castanea sativa* Mill.). Chestnut blight disease can be controlled by means of a biological method. This biological control procedure is based on virulent and hypovirulent forms of the fungus *C. parasitica* that occur in the nature.
The first application of a biological programme in Europe for the control of the chestnut blight, using hypovirulent strains of the pathogen was conducted in southern France in the years from 1967 to 1972. It yielded surprisingly positive results. These were the first significant successes in attempting to control this important disease (Grente and Berthelay-Sauret 1969, 1979).
The aim of this study was to treat cankers with hypovirulent strains converted with French (INRA Clermont Ferrand) and Hungarian hypoviruses and to evaluate the efficiency of biological control in the monitored localities.

Materials and Methods

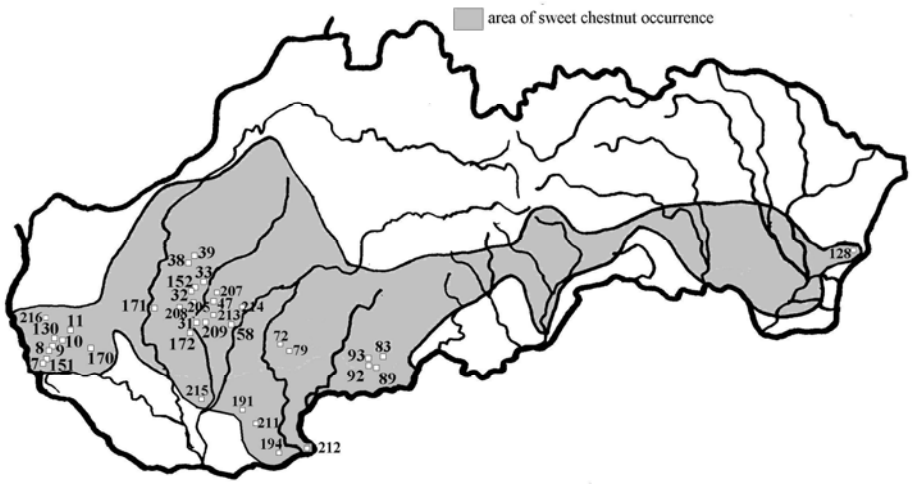
Treatments were performed on stems and branches of trees infected by virulent strains of the fungus. Inoculation holes (5-8 mm x 5-10 mm, depending on the bark thickness) were made using an auger with flat edges so as to establish close contact between the hypovirulent mycelium of the fungus and the virulent mycelium causing the canker. Pellets, containing the hypovirulent mycelium, prepared specifically for our trials by the Fytofarm (Phytofarm) of Bratislava, according to Grente (1965), were applied directly into the inoculation holes. Cubes of malt agar with hypovirulent mycelium were also used. The holes were then sealed with grafting wax.
Nontreated cankers in all localities (on the same stems and also on separated trees) were used as controls to compare the effect of biological control and to assess the natural spread of hypovirulence. The effect of treatment was

evaluated visually (callus formation; enlargement of cankers outside the line of treatment). The size (width and length) of treated and nontreated cankers was measured in mm. The degree of healing of the cankers was evaluated once every year after the treatment.
As no hypovirulent isolates were previously detected in Slovakia, virulent isolates of *C. parasitica* from Slovakia were converted into hypovirulent forms with French hypovirulent isolates maintained in the «INRA-Station d´Agronomie et Mycologie, Unité de Mycologie in Clermont Ferrand». Four new hypovirulent isolates were thus obtained. These new hypovirulent isolates (with dsRNA) were designated TchA, TchD, TchC and TchE (Juhásová and Berthelay-Sauret 1993).
Slovakian virulent isolates were also transformed into hypovirulent forms with Hungarian hypovirulent isolates (R5, C2, IHB2) and were used for biological control.

Results and Discussion

European chestnut occurs in 5 subregions in 210 localities in Slovakia. Chestnut blight was identified in 36 localities (Figure 1/A-B).
In 1992, only normal cankers (i.e. infected with virulent strains) were identified in the studied localities. The number of cankers varied from 1 to 15 per tree. Also, new cankers continued to form on the study trees. The results of the biological control using French hypovirus containing strains performed in the localities are summarized in Table 1. The treated cankers occurred on the stems and on branches. A total number of 4173 cankers were treated within the 1992-1997. During the course of the study, from 22.6 to 68.5% of all the treated cankers were callused and callusing has been started on the remaining treated trees.
Treatment with strains converted with Hungarian hypovirulent strains was done in two localities (Bratislava, Modra) in 2005 (Tables 2, 3). The evaluation of the success of the biological control programme will be realized in this autumn.
In Hungary, a similar biological programme was initiated in two localities (Ágfalva and Zengővárkony) in 1997, where Hungarian hypovirulent strains were used. The rate of callusing of the treated cankers in these two localities was 80-90%. Compared to the results of our first attempt to use hypovirulent strains in Slovakia, the study in Hungary was more successful (Radócz, 2001). Indeed, in our first tests the number of callusing cankers was not more than 50%.

A



B

Locality	No. of locality	Locality	No. of locality
Arborétum Mlyňany	58	Pastúchov	208
Bojná	33	Petrovce	128
Bratislava	7	Pezinok	10
Duchonka	39	Podhájska	191
Grinava	9	Podhradie	38
H. Lefantovce	47	Príbelce	89
H. Plachtince	93	Rača	151
Hlohovec	171	Radošina	32
Chľaba	212	Senec	170
Kovarce	204	Solčany	213
Krnča	207	Stredné Plachtince	92
Limbach	130	Súlovce	214
Lipovník	152	Svätý Jur	8
Modra	11	Svodín	211
Modrý Kameň	83	Štitáre	209
Nitra	31	Štúrovo	194
Nitrianska Streda	205	Tlstý Vrch	79
Žemberovce	72	Veľké Lovce	215

Figure 1/A-B. The map (A) and localities (B) of occurrence of *C. parasitica* in Slovakia in 2005

Up to the year 2002, no hypovirulent strains of *C. parasitica* were isolated in Slovakia. However, in that year, we found cankers showing callus on trees which has not been treated so far. It thus appeared the hypoviruses would have spread to the strains of *C. parasitica* causing the cankers. Hypovirulent strains grew and sporulated so poorly that their persistence was limited. A combination of several favourable factors may be involved in the natural spread of hypovirulence. The sporulation of hypovirulent strains is much lower than the sporulation of virulent strains of *C. parasitica*. As hypoviruses may be present in conidia and not in ascospores (Bissegger et al., 1997), transmission of hypovirulence is mostly by means of conidia. However, about 10 - 50% of the conidia produced by hypovirulent strains do not contain the hypovirus and these can give rise to virulent mycelium (Anagnostakis, 1995).

Table 1. Results of biological control of chestnut blight disease with converted strain containing French hypovirus in Slovakia within the 1992-1997

Locality	**Number of evaluated trees**		**Number of cankers**	
	Total	**Infected**	**Treated**	**Callusing (%)**
Bratislava	2847	88	238	44.2
Svätý Jur	2361	59	103	49.9
Myslenice	585	61	96	51.6
Rača	1144	181	227	45.2
Modra	890	110	176	63.2
Limbach	878	138	490	68.5
Duchonka	2429	693	1476	51.8
Lipovník	1030	1166	492	52,9
S. Plachtince	3063	313	375	22.6
H. Plachtince	1092	82	158	32.7
M. Kameň	4962	97	181	42.7
Radošina	1863	158	158	45.4

Table 2. Results of treatment of cankers on European chestnut with converted hypovirulent isolates (Hungarian hypovirus) of *C. parasitica* in Bratislava-Sliačska in 2005

No. of tree	Trunk in girth	Hypovirulent isolate	Treatment on trees		
			Trunk basis	Trunk	Branches
1	348	BA-SL/C	-	-	+
2	322	BA-SL/C	-	+	-
3	462	BA-SL/C	-	+	+
4	278	BA-SL/C	+	+	+
5	297	BA-SL/C	-	-	+
6	309	BA-SL/C	-	+	-
7	219	BA-SL/C	+	+	+
8	176	BA-SL/C	+	+	+
9	175	BA-SL/C	+	+	+
9a	14	BA-SL/C	-	+	-
9b	12	BA-SL/C	-	-	-
10	215	BA-SL/C	+	+	-
10a	18	BA-SL/C	-	+	-
11	72	BA-SL/C	+	+	+
12	185	BA-SL/C	+	+	+
13	255	BA-SL/C	-	-	+
14	420	BA-SL/C	+	+	+
15	220	BA-SL/C	+	+	+
16	132	BA-SL/C	+	+	+
17	62	BA-SL/C	+	+	+
18	270	BA-SL/C	-	-	-
19	210	BA-SL/C	+	+	+
20	120	BA-SL/R5	+	+	+
21	40	BA-SL/R5	-	+	+
22	330	BA-SL/R5	+	+	+
23	330	BA-SL/R5	+	+	+
24	85	BA-SL/R5	-	-	+
25	220	BA-SL/R5	+	+	+
25a	80	BA-SL/R5	+	+	+

Table 3. Results of treatment of cankers on European chestnut with converted hypovirulent isolates (Hungarian hypovirus) of *Cryphonectria parasitica* in Modra – Gaštanka, in 2005

No. of tree	Trunk in girth	Hypovirulent isolate	Treatment on trees		
			Trunk basis	Trunk	Branches
1b	26	Mo-R5x2	-	+	-
2	36	Mo-R5x2	+	-	+
6	36	Mo-R5x2	+	-	-
6a	32	Mo-R5x2	-	+	-
6b	38	Mo-IHB2	+	+	+
9	36	Mo-IHB2	-	+	-
13	135	Mo-IHB2	+	+	+
16b	71	Mo-R5x2	+	+	-
20	79	Mo-R5x2	-	+	+
27c	56	Mo-IHB2	+	+	+
29	65	Mo-IHB2	+	+	+
30b	64	Mo-IHB2	+	+	+
41a	25	Mo-IHB2	-	+	-
45	218	Mo-R5x2	-	+	+
54a	207	Mo-IHB2	+	+	+
55	62	Mo-IHB2	+	-	+
58b	140	Mo-IHB2	+	+	+

Acknowlegment

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RESULTS OF THE USE OF *CRYPHONECTRIA PARASITICA* HYPOVIRULENT STRAINS IN HUNGARY AND IN SLOVAKIA

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Summary

Chestnut blight disease can be controlled by means of a biological method using hypovirulent strains of the pathogen. Chestnut blight was identified in 36 localities in Slovakia. Cankers were treated with hypovirulent strains converted with French (INRA Clermont Ferrand) and Hungarian hypoviruses. A total of 4173 cankers were treated within the 1992-1997. During the study from 22.6 to 68.5% of all the treated cankers were callused and callusing has been started on the remaining treated trees. Up to the year 2002, no hypovirulent strains of *C. parasitica* were isolated in Slovakia. However, in that year, we found cankers showing calluses on trees which had not been treated earlier. Natural spread of hypovirulence in Slovakia has been presented.

CHESTNUT BLIGHT INFECTION ON SESSILE OAK (*QUERCUS PETREA*) IN SOUTHERN-HUNGARY

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"Chestnut blight" disease caused by *Cryphonectria parasitica* (Murr.) Barr (syn: *Endothia parasitica* [Murr.] And.) causes big damages of the chestnut stands throughout the world. First at the beginning of the XX-th. century it destroyed almost the whole American chestnut (*Castanea dentata*) populations in the USA (Anagnostakis, 1987). In the middle of the XX-th century the pathogen was transferred into Europe and infected the European chestnut (*Castanea sativa*) populations in the West-European countries. This infection was reported first in Italy, near Genova in 1938 (Biraghi, 1946).

Then the disease spread from Western-Europe towards the Central- and Eastern-European chestnut territories and arrived to the Carpathian-Basin too. "Chestnut blight" disease symptoms were reported on chestnut first in Austria (Donaubauer, 1964), in Hungary (Körtvély, 1970), in Slovakia (Juhasova*,* 1976), in Romania (Florea*,* Popa, 1989) and in Ukraine (Radócz, 2001). Chestnut stands in Hungary have already been seriously dameged by this disease at the end of last century (Radócz*,* 1999).

This fungus is the most important disease for European chestnut, but the importance of this parasite is increased because it is able to infect other tree species of the *Fagaceae* family (oak, beech). Until 1998 the fungus was only detected on *Castanea sativa* in Hungary. Then the blight symptoms were also detected on some *Quercus petrea* trees in mixed chestnut forest near Kőszeg and Zengővárkony (Radócz and Holb, 2002). Although symptoms were not so serious in *Quercus* spp. than *Castanea* spp., it seems that *Cryphonectria parasitica* frequently threatens the young Hungarian oak trees mainly in infected chestnut forests. Therefore it could be a more serious potential parasite for our forests. One of the most important species of the Hungarian forestry is the sessile oak (*Quercus petrea*) which has been threatened by *Cryphonectria parasitica*. These oak trees are important both economically and ecologically aspects.

Literature

Chestnut blight fungus (teleomorph: *Cryphonectria parasitica* (Murrill) Barr [syn.: *Endothia parasitica* (Murr.) P.J. Anderson and H.W. Anderson];

anamorph: *Endothiella* sp.) is an ascogenous pathogen causing blight on some species of the family *Fagaceae* (*Castanea* spp., *Fagus* spp., *Quercus* spp.).

Symptoms: On young stems, brown lesion forms on the smooth bark can be found. Some discoloration occurs also when older stems are attacked, mainly through wounds. Lesions become sunken as the bark and the cambium are killed; there is swelling and cracking of the outher bark. Death of the cambium of this ring-porous tree prevents formation of the xylem vessels needed for liquid transport, and this causes wilting of the leaves above (beyond) the canker. Pycnidia are very abundant on the cancered bark, exuding spore tendrils in moist conditions. Pale brown, mycelial fan formations can be found in the inner bark (Sivanesan and Holliday, 1981).

Appearance on* Quercus *spp.: *C. parasitica* caused bark necrosis on some species of the genus *Quercus* (Torsello et al., 1994) in the USA and in a few European countries. They reported that 15.5 % of the oaks in Pennsylvania (mountain regions) were infected with the chestnut blight fungus. In Hungary, the fungus was detected first on oak at Zengővárkony and Kőszeg (Radócz and Holb, 2002). Symptoms were never higher in the infection scale than disease grade 2 and dead parts of some of the oak branches due to this parasite were observed. In 2003 infected oak trees with bark necrosis were also found in Baie Mare, Romania. Laboratory identification showed that *Quercus* spp. trees were infected by some of the same *C. parasitica* strains that were detected from chestnut. It is supposed that *Quercus petrea* infections were caused by the inoculum originated from fruit bodies of chestnut bark cankers.

Materials and Methods

Main goals of our studies were the followings: to estimate the damages caused by the *Cryphonectria parasitica* fungus on oak trees; laboratory investigation and identification of the collected samples and isolates.

Our field examinations has been made at Bakonya (Mecsek-Mountain, Southern-Hungary) in a young sessile oak plantation mixed with chestnut several times, since 2003. At the beginning of the investigation we selected a sample-field with 150 oak trees. The trees were 7-8 years of age with 2-5 m heights as the average. During the field examinations every trees were checked. We found the symptoms of *C. parasitica* and we measured the infection rates according to Table 1. Bark samples were collected from the infected or suspicious looking trees by a disinfected sharp scalpel for further laboratory identifications.

Table 1. Infection rate classification system

Degrees of contamination		
1		Healthy tree
2		Suspicious symtom
	a	in the crown of the tree
	b	on the trunk of the tree
3		1 cancer
	a	symptoms in the crown
	b	on the trunk
4		More cankers
	a	symptoms in the crown
	b	on the trunk
5		Killed tree by *C. parasitica*

During the laboratory examinations PDA (potato-dextrose-agar) media were used. Surfice sterilized bark samples were cultivated on PDA media and the isolates were incubated during 7 days in a climated chamber. After this cultivation *Cryphonectria parasitica* was identifiable on the media.

Results and Discussion

Field examinations has done yearly, since 2003 (in 2004, 2005, 2006). We learned that chestnut blight disease was spread through the territory, and symptoms were observable well on several trees. Infection rate examinations were done during every occasions. We can see the result of the yearly examinations on Table 2. It was detected that *C. parasitica* infection is more and more serious year by year.
Previous statement had been verified during the laboratory examinations, that is chestnut blight fungus infected several young oak trees on the sample-field as it is shown in Table 3.

Table 2. Results of infection rate in field examinations at Bakonya

	Infection rate		
Time of field examination	**2004. 12. 07.**	**2005. 11. 10.**	**2006. 09. 28.**
Tree number			
BAK. 1.	-	2b	2ab
5.	-	2b	-
6.	2b	3b	4a
7.	5	5	5
8.	-	4ab,	5

Table 2. (continued)

10.	-	2b	-
12.	-	2b	2ab
14.	2b	2b	-
17.	2b	2b	2ab
19.	2b	2b	2b
20.	-	3b	3b
21.	2b	-	2b
23.	3b	4b	3ab
24.	4b	4b	4ab
30.	-	-	2ab
31.	-	-	2b
33.	-	-	3ab
34.	2b	2ab	2ab
35.	3b	3a	3ab
38.	4ab	4ab	4ab
41.	3b	5	5
43.	2b	-	-
49.	3b	3b	3ab
50-	-	-	2ab
52.	2b	-	-
53.	4b	4ab	5
55.	5	5	5
56.	4ab	4ab	4ab
62.	-	2b	3ab
63.	-	3b	3ab
65.	-	2b	-
66.		2b	2b
67.	2b	3b	3b
68.	-	2b	-
73.	4b	4b	4ab
80.	-	2b	4a
81.	-	3b	4b
86.	-	3b	4ab
87.	2b	3b	3ab
88.	4b	4ab	4ab
89.	-	-	3ab
91.	5	5	5
93.	-	2b	3ab
94.	2b	-	-
96.	-	2b	2b
97.	2b	3b	3ab
99.	-	4ab	5
100.	2b	3b	3b
109.	4b	4b	4ab
110.	3b	3b	3ab
116.	4b	3b	3b
118.	3b	4ab	4ab
119.	5	5	4ab
126.	3b	3b	3ab
132.	4b	4b	4ab
134.	2b	-	-
137.	5	5	5
138.	2b	-	-
145.	3b	4ab	4ab
148.	2b	2b	2a
149.	2b	3b	3ab
150.	4b	4ab	4ab

Remark: Trees signed by numbers missing from the Table 2 were found healthy

Table 3. Results of *C. parasitica* identification on laboratory examinations

Infected isolates by *C. parasitica* according to the latest laboratory test (2005 November)
BAK 6, 7, 8, 17, 20, 23, 24, 34, 35, 38, 41, 49, 53, 55, 56, 63, 67, 73, 80, 81, 86, 87, 88, 91, 93, 97, 99, 100, 109, 110, 116, 118, 119, 126, 132, 137, 145, 148, 149, 150

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CHESTNUT BLIGHT INFECTION ON SESSILE OAK (*QUERCUS PETREA*) IN BAKONYA

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Summary

Cryphonectria parasitica causes big damages of the chestnut-stads throughout the world. The pathogen arrived to the Carpathian-Basin in the second part of the last century and destroyed the chestnut populations. Later the symptoms of the disease appeared on sessile oak trees in oak-chestnut mixed forests in Hungary and in other countries. The goals of our studies were to estimate damages caused by *Cryphonectria parasitica* on oak trees in a young sessile oak plantation mixed chestnut and to investigate the collected samples and isoletes in laboratory. Our results showed that chestnut blight fungus infected several young oak trees on the oak plantation in Bakonya. Therefore this disease is a new serious potential danger for our oak forests.

DISTRIBUTION OF TRANSPOSONS IN *BOTRYTIS CINEREA* ISOLATES COLLECTED FROM THE WINE REGIONS OF EGER AND TOKAJ, HUNGARY

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Botryotinia fuckeliana (de Bary ex de Bary) Whetzel (anamorph: *Botrytis cinerea* Pers.:Fr.) is a cosmopolitan ascomycetous fungus that causes grey mould on a great number of plants in the temperate zone worldwide by infecting various tissues (Jarvis, 1980). In grapevine, the frequent occurrence of *B. cinerea* prior harvesting results in serious losses of fruits and deterioration of wine quality. This is also the case in Eger, a major Hungarian wine region in the North-Eastern part of the country, where *B. cinerea* is considered to the third most important grapevine pathogen after downy mildew (*Plasmopara viticola* /Berk. and Curt ex de Bary/ Berl. and de Toni) and powdery mildew [*Erysiphe necator* Schwein. var. *necator* (syn.: *Uncinula necator* /Schwein./ Burrill var. *necator*)], with an estimated annual loss of up to 15-20 %. In contrast, some 100 km eastwards in the Tokaj wine region, *B. cinerea* is also responsible for the phenomenon called 'pourriture noble' (noble rot). Under certain unique environmental conditions, mycelia growing on the surface of the uninjured, healthy berry drains water (but no substrates) via the fine infection hyphae. As a consequence, the concentration of all the soluble compounds within the berry significantly increase (Jarvis, 1980). Such berries yield the sweet, special quality wine called „aszu".

Literatute

Transposable elements (TEs) are fragments of DNA that can insert into new chromosomal locations and often make duplicate copies of themselves in the process (Feschottes et al., 2002). TEs were first discovered in maize (McClintock, 1984) and later have been found in several eukaryotic, eubacteria and archaea genome. In addition to the wide array of 'hosts', the variety of transposons described also increased considerably (Finnegan, 1989). Fungal transposons were first identified in the yeast *Saccharomyces cerevisiae* (Boeke, 1989), though the first indirect evidence for their

presence in filamentous fungi arose years earlier from conventional genetic studies with *Ascobolus immersus* mutants (Decaris et al., 1978).
There are two main classes of TEs (Finnegan, 1989). Class I elements are related to retroviruses and they transpose through the reverse synthesis of DNA from template RNA, while class II elements move in the genome through direct DNA to DNA transposition without an RNA intermediary. Class I elements are known as retrotransposons and include TEs with or without 'long terminal repeated sequences' (LTRs). Retroelements have been found in a number of fungal species such as *Alternaria alternata* (Kaneko et al., 2000), *Ascobolus immersus* (Goyon et al., 1996), *Aspergillus fumigatus* (Neuvéglise et al., 1996), *Aspergillus nidulans* (Nielsen et al., 2001), *Neurospora crassa* (Kinsey and Helber, 1989). DNA transposons are also widespread and have been described among others in *Agaricus bisporus* (Sonnenberg et al., 1999), *Ascobolus immersus* (Colot and Rissignol, 1995), *Aspergillus niger* (Glayzer et al., 1995), *Magnaporthe grisea* (Kachroo et al., 1994), *Nectria haematococca* (Enkerli et al., 1997), *Neurospora crassa* (Yeadon, 1995), and *Podospora anserina* (Hamann et al., 2000).
B. cinerea has been shown to possess two transposons. *Boty* is a class I LTR-retro-transposon (Diolez et al., 1995), while *Flipper* is a class II element (Levis et al., 1997). In this paper we will show that at least four genotypes of isolates related to the presence or absence of these transposons occur in the Eger wine region. Potential significance of this finding is discussed.

Materials and Methods

Field strains of *B. cinerea* were collected from various locations of the Eger and Tokaj wine districts. They were isolated from infected berries between 2003 and 2004 during the vintage period (September-October). Fungal strains from both wine regions are numbered by the chronology of collection, irrespective to the local provenance.
DNA was extracted from aerial mycelium of *B. cinerea* with Plant DNA Purification Kit (QuiaGene). Transposons were detected with PCR reactions described at Munoz et al., 2002. Presence or absence of the two transposons was confirmed by agarose gel electrophoresis (Figure 1) using standard protocols (Sambrook et al., 1989).

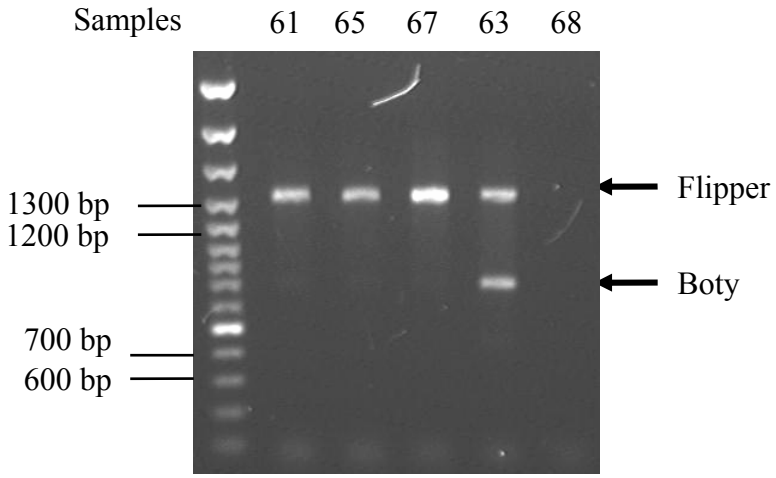

Figure 1. Gel electrophoresis picture of transposon elements

Results

B. cinerea has been shown to possess a highly versatile genome reflected in a considerable metabolic flexibility. Depending on the actual environmental conditions (Martinez et al., 2003), the fungus is able to act both as a saprophyte and a pathogen, and has developed resistance to most of the fungicides used to control it (Faretra and Pollastro, 1991; Leroux et al., 1999).

Studies on French and Chilean *B. cinerea* isolates have revealed the presence of three distinct intrapopulation: (1) transposa, having transposable elements *Boty* and *Flipper*, (2) vacuma, having none of the two and (3) boty containing the transposable element *Boty* alone (Munoz et al., 2002). The flipper intrapopulation, containing the transposable element *Flipper* alone has thus far only been described in two separate isolates from the United Kingdom and France (Albertini et al., 2002).

Boty and *Flipper* are both specific for *B. cinerea*. While proteins encoded by transposable elements are supposed to be used exclusively for the purpose of their own reproduction without any interference with the metabolism of the host organism, a more rapid biomass formation in the vacuma intrapopulation relative to boty and transposa was observed (Martinez et al., 2003). It should also be noted that the level of fungicide resistance significantly differed in transposa and in vacuma-type *B. cinerea* populations in French isolates (Albertini et al., 2002). It is not yet known whether the two events are related to each other, and if they are then it is a cause or a consequence of the altered transposon pattern.

In the framework of this project 68 and 17 *B. cinerea* isolates have been collected from the Eger and Tokaj wine regions, respectively. To the best of

our knowledge, this is the first Central-Eastern European collection of its kind, and only the fourth worldwide. Two French studies from the Champagne and Bordeaux wine regions, respectively analysed a collection of 259 (Giraud et al., 1997) and 121 (Martinez et al., 2003) isolates, while a Chilean study was based on 69 cases. Distribution of the *Flipper* and *Boty* transposons in the Hungarian isolates are markedly different to those in French and Chilean collections (Table 1).

Table 1. Distribution of transposons in *Botrytis cinere*a isolates collected from the Eger and Tokaj vine regions, Hungary, and percentage of the transposa, vacuma, flipper and boty intrapopulations in other countries as found in the literature

Type of strain[a]	No. of isolates[b]		Percentage		Percentage		
	Eger	Tokaj	Eger	Tokaj	Chile[(c)]	France[(d)]	France[(e)]
transposa	12	0	17.64	0	79.71	75.00	61.34
flipper	51	17	75.00	100	0.00	0.00	0.00
boty	1	0	1.47	0	11.59	0.00	0.00
vacuma	4	0	5.89	0	8.70	25.00	38.66
All	68	17	100	100	100	100	100

[a] transposa isolates contain both the *Boty* and *Flipper* transposable elements. Flipper and boty are isolates containing *Flipper* or *Boty*, respectively, while vacuma are isolates without *Boty* and *Flipper*.
[b] all the isolates as well as viticultural and geographical details of their provenance are available from the first author of this paper upon request.
[c] Munoz et al.,2002; [d] Giraud et al., 1997; [e] Martinez et at., 2003

While genotype transposa is clearly prevalent in the French and Chilean samples with some two-third of the isolates containing both transposons, the percentage of this particular intrapopulation was less than 18 percent in Eger and zero percent in Tokaj. While genotype boty was present in over 10 percent of the Chilean collection, we found only one single strain in Eger (and none in Tokaj) that carried the *Boty* transposon alone. We note, while the French studies indicated the presence of boty genotype in their collections, no defined values were provided in either case.
Distribution of the genotype vacuma ranged between 8 and 38 percent in the literature. Our investigations yielded only a handful of vacuma isolates in Eger and none in Tokaj.
The most striking observation in our study is the appearance of the genotype flipper, an intrapopulation of *B. cinerea* hitherto considered extremely rare. However, this genotype is obviously prevalent in the two Hungarian wine regions studied, with 75 percent of the isolates containing only flipper

transposon in Eger and all in Tokaj. None of the three collections cited in this paper have reported on the appearance of this genotype.
Transposons are highly mobile genetic elements while *B. cinerea* is a truly cosmopolitan fungus. Comparative analysis of transposon distributions in *B. cinerea* isolates collected from all around the world may thus be a worthy method to study fungal population genetics.
There are no tested hypothesises on the physiological role of transposons. Mobility of transposons including those in the filamentous fungi *Magnaporthe grisea* (Ikeda et al., 2001) and *Fusarium oxysporum* (Mes et al., 2000) were reported to increase during certain stress conditions such as substrate deficiency, drought, heat and exposure to γ-radiation. It remains to be tested whether stress conditions will influence *B. cinerea* transposons in anyway.

Discussion

This study showed that all of the four transposon-related genotypes of *B. cinerea* ever described in the literature exists in the Eger wine region. Most noteworthly, genotype flipper, considered extremely rare elsewhere in the world is apparently dominant both in the Eger and Tokaj wine regions. In fact in Tokaj, flipper was the only transposon found. It remains to evaluate whether *B. cinerea* genotypes defined over transposon distribution are relevant to the role the fungus plays in viticulture and enology.

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DISTRIBUTION OF TRANSPOSONS IN *BOTRYTIS CINEREA* ISOLATES COLLECTED FROM THE WINE REGIONS OF EGER AND TOKAJ, HUNGARY

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Summary

Analysis of the distribution of the transposable genetic elements Boty and Flipper in *Botrytis cinerea* (grey mould) isolates collected from the Eger and Tokaj wine regions, North-Eastern Hungary is presented. We demonstrate the prevalence of a rare intrapopulation called Flipper, and discuss the differences among *B. cinerea* populations isolated from Western European and South American wine regions.

STUDIES OF EVOLUTIONARY RELATIONSHIPS OF *PHOMA* SPECIES BASED ON PHYLOGENETIC MARKERS

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Introduction

Phoma is a cosmopolitan genus of coelomycetous fungi. Many species have been reported from wide range of hosts, substrates, particularly as pathogens from plants, as well as soil-borne but predominantly saprophytic and opportunistic species have also been isolated. Almost 2000 *Phoma* species have been reported throughout the world till now (Boerema et al., 2004).

There are several ways in the traditional and modern mycology to contribute to taxonomical studies of fungi including morphology, biochemistry, nucleic acid sequences and many others.

The three most commonly discussed species concepts are morphological, biological, and phylogenetic ones. Since the beginning of mycology, studies of species concept in fungi have been mainly based on morphological elements. The most of the species and other taxa of *Phoma* have so far been determined on the basis of morphology on standardized media, and gene sequence analysis was only used as a confirmative or distinctive complement. Thus, members of the genus are primarily defined by the application of the Morphological Species Recognition (MSR). The weakness of MSR is that species diagnosed by this often comprise more than one species when diagnosed by Biological Species Recognition (BSR) or Phylogenetic Species Recognition (PSR). Biological species concept defines species as groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups (Mayr, 1942). BSR is acceptable for many fungi, where sexual reproduction occurs. But there are also fungal groups, where sexual reproduction has never been discovered. Approximately 20% of fungi are morphologically asexual and do not produce meiospores (Reynolds and Taylor, 1993). Since strains of *Phoma* spp. apparently can not be crossed, the application of the BSR concept is impracticable. Though, despite reproducing asexually, many anamorphic fungi including *Phoma* spp. are known to possess a surprisingly high level of genetic variation (Khon, 1995; Talhinhas et al., 2002).

The current advances in biochemical and molecular research have provided mycologists with powerful tools that can be used for delineation of fungal taxa. The PSR defines species as the smallest aggregation of populations with a common lineage that share unique, diagnosable phenotypic characters (Harrington and Rizzo, 1999). According to Taylor et al. (2000) seems to be well suited for fungi and likely to become very popular with mycologists, because it can be applied equally both to sexual and to asexual organisms. Taylor et al. (2000) proposed the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) *for species defining,* which *could be* an attractive alternative or complement to the morphological species concept, but has not been widely applied to *Phoma* spp. yet. It requires the analysis of several unlinked genes and implies that the phylogenetic position of a true species is concordant in at least some of them and can not be contradicted in the others.

Up to now the characterization of *Phoma* species has been mostly applied on the basis of morphology, phenotype and physiology. Recently, Boerema et al. (2004) published *Phoma* Identification Manual, based on morphological studies which contains 223 cultural descriptions of specific and intraspecific taxa of *Phoma* Sacc.

In the middle of 90s, due to the advances in molecular and biochemical research of that time molecular markers were identified in *Phoma*. Some isozyme analyses were applied to distinct some morphologically identical *Phoma* species from each other (Kövics and Gruyter, 1995). Protein polymorphisms comparing to DNA polymorphisms is unfavorable, because protein electrophoresis assays the genotype indirectly, and a high proportion of the variation occurs at the DNA level may not be detectable as it does not alter the amino acid composition of the protein. Similarly, some changes in amino acid composition do not change the electrophoretic mobility of the protein, and remain undetected, leading to different genotypes being assigned to the same allozyme allele.

DNA polymorphisms are based on differences in DNA sequences and have three enormous advantages over protein polymorphisms. The first is, that the sequence differences are detected directly. The second advantage is that they occur in a genome at very high frequency, and finally, they are not subject to selection pressure, in case they do not affect the phenotype. But morphological characterization besides molecular tools will remain a basic and powerful key in the identification of *Phoma* species.

One of the most commonly used molecular techniques for assessing phylogenetic relationships is to evaluate the sequences of certain fungal DNA regions. Phylogenetic sequence comparisons concentrate on a comparison of the coding portions of the ribosomal genes and their RNA products, allowing discrimination at different taxonomic levels. Many phylogenetic works are based on the internally transcribed spacers (ITS),

which are one of the most widely used molecular markers due to their highly variability in nucleotide sequences.

According to Lutzoni et al. (2004) 83.9% of fungal phylogenies are based exclusively on sequences from the ribosomal RNA tandem repeats. Because of it, there is a consequent trend toward inclusion of other gene loci in the data sets, gathered for phylogenetic analysis. Among these genes, protein-coding genes like β-tubulin and translation elongation factor (*tef1*) can contribute greatly to resolving deep phylogenetic relationships with high support and/or increase support for topologies inferred using ribosomal RNA genes.

In this study we have obtained DNA sequences from ITS and translation elongation factor coding genes to resolve phylogenetic relationships among several *Phoma* species, since it has been shown that usage of multigene datasets can increase the resolution of molecular phylogenetic analyses.

Ribosomal DNA (rDNA) has long been used as a potential marker for phylogenetic studies (reviewed in Avise, 2004). rRNA genes are organized in clusters of tandemly repeated units, each of which consists of coding regions (18S, 5.8S, and 28S; Gerbi, 1985) and 2 internal transcribed spacers (ITS) and intergenic spacer (formerly called the „ Non-Transcibed Spacer, NTS) region. While the coding regions are evolutionarily conserved and have been utilized for phylogenetic inferences for major phyla (reviewed in Hills and Dixon 1990), the 2 ITS regions are appropriate for detecting differences between co-specific individuals and are hence potentially useful markers to study the relationships of populations and closely related species in fungal, plant, and animal taxa due to their relatively rapid evolutionary rates (Baldwin, 1992; Schlötterer et al., 1994; Mai and Coleman, 1997; Weekers et al., 2001; Oliverio et al., 2002; Chen et al., 2000, 2002). In this study we have obtained a region of nuclear rDNA, containing the internal transcribed spacer regions 1 and 2 and the 5.8S rDNA (Figure 1).

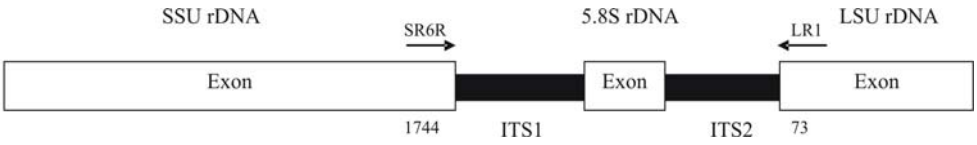

Figure 1. Schematic structure of ITS region in *Phoma* spp. and location of primers for phylogenetic analyses

Translation elongation factor 1 subunit alpha (EF1α=*tef*1) is part of the cytosolic EF1 complex, whose primary function is to promote the binding of aminoacyl-tRNA to the ribosome in a GTP-dependent process (Moldave, 1985). It is an essential component of the protein synthesis process in eukaryotes and archeabacteria. Complexed with GTP, it carries the aminoacyl-tRNA to the A site of the ribosome-mRNA–peptidyl-tRNA complex; upon hydrolysis of GTP it leaves the ribosome as EF-1α-GDP.

Simultaneously, elongation factor 1α (EF-1α) is a highly conserved ubiquitous protein that has been suggested to have desirable properties for phylogenetic inference (Roger et al., 1999). EF-1α is well suited for determining phylogenetic relationships, due to its universal occurrence and presence typically as a single copy within the genome (Baldauf and Doolittle, 1997). It has been proven to be a useful gene to resolve phylogenetic relationships at species level as well as in deeper divergences where amino acid substitutions provide phylogenetic resolution. Here we have used primer pair which facilitates the PCR amplification of the large intron of tef1 gene (Druzhinina and Kubicek, 2005, Figure 2).

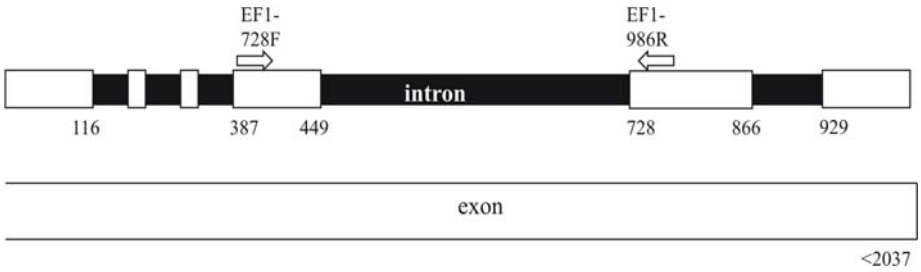

Figure 2. Schematic structure of *tef1* gene in *Phoma* spp. and location of primers for phylogenetic analyses

Materials and Methods

Twelve isolates of eleven *Phoma* species were tested for phylogenetic analyses in this present study (Table 1). All isolates were identified morphologically according to Boerema et al. (2004) based on physiological and morphological characteristics.

Mycelium from isolates were transferred to 100 ml Erlenmayer flasks containing 50 ml malt broth (2% malt exract). The cultures were grown at room temperature for 48 hours in the dark on a rotary shaker (125rpm). The mycelium was harvested by vacuum filtration. Total genomic DNA was extracted from freeze-dried mycelium and isolated using the E.Z.N.A.® TM

Fungal DNA Isolation Kit (Omega Bio-tek, Inc., USA) according to the protocol (as following the manufacture instructions).

Primers used to amplify the ITS region containing the internal transcribed spacer regions 1 and 2 and the 5.8S rDNA are based on published composite sequences, SR6R and LR1 (White et al., 1990) with the following amplification protocol: 3 min initial denaturing at 95°C, followed by 5 cycles of 1 min at 95°C, 1 min annealing at 50°C, 1 min at 72°C and 25 cycles of 1 min at 90°C, 1 min annealing at 50°C, 1min at 72°C and 15 min final extension at 72°C. The large intron of the *tef1* gene was amplified by the EF1-728F and EF1-986R primer pair (Druzhinina and Kubicek, 2005) according to the following program: 3 min initial denaturing at 95°C, followed by 5 cycles of 1 min at 95°C, 1 min annealing at 59°C, 1 min at 72°C and 25 cycles of 1 min at 90°C, 1 min annealing at 59°C, 1min at 72°C and 15 min final extension at 72°C. Purified amplification products were sequenced by MWG Biotech Company in Germany.

The obtained DNA sequences were aligned first with ClustalX (Thompson et al., 1997) and manually adjusted using Genedoc (Nicholas et al., 1997). Single gaps were treated either as missing data or as the fifth base and multistate characters were treated as uncertain.

Phylogenetic analyses were performed in PAUP*4.0b (Swofford, 2002). The following settings were used: heuristic search with tree bisection-reconnection (TBR), with random addition of sequences with 1000 replicates. Stability of clades was assessed with 1000 bootstrap replications.

Results

Twelve isolates of eleven *Phoma* species were compared in this study. The morphological identification of the isolates was done following the descriptions of Boerama et al. (2004). The obtained results indicated that the microscopical and cultural characteristics of the concerned *Phoma* isolates fit to the identity of *Phoma* species given in Table 1.

Table 1. Isolates of *Phoma* species

Species	Isolate number		Host of origin
	Our collection	**Original**	
Phoma eupyrena	D/058	CBS 375.91	*Phaseolus vulgaris*
Phoma destructiva	D/033	?	*Lycopersicon esculentum*
Phoma pinodella	D/035	D/035	*Glycine max*
Phoma foveata	D/048	PD 76/1021	*Chenopodium quinoa*
Phoma herbarum	D/143	MTCC 2319	?
Phoma exigua var. *exigua*	D/075	D/075	*Glycine max*
Phoma exigua var. *exigua*	D/077	D/077	*Glycine max*
Phoma exigua var. *linicola*	D/071	PD 86/73	*Linum usitatissimum*
Phoma glomerata	D/034	D/034	*Glycine max*
Phoma multirostrata	D/044	PD 77/508	*Phylodendron* sp.
Phoma plurivora	D/072	PD 75/907	*Medicago sativa*
Phyllosticta sojicola (=*Phoma exigua* var. *exigua* ?)	D/050	CBS 301.39	*Glycine max*

Translation elongation factor

We amplified and sequenced a 0.2 kb fragment of the large intron of the *tef1* gene from twelve isolates of eleven *Phoma* species and subjected it together with the ITS1-5.8Sr-DNA-ITS2 to a combined parsimony analysis with PAUP.

For phylogenetic analyses of *tef1* fragments we involved other *Phoma* and *Ascochyta* species as well as *Claviceps* and *Leptosphaeria* species as outgroup, all were downloaded from GenBank maintained by the NCBI (Table 2). *Didymella fabae* and *Didymella lentis* are the teleomorph of *Ascochyta fabae* and *Ascochyta lentis*, (Kaiser et al., 1997).

Table 2. Species involved in the phylogenetic analyses of *tef1* fragments

Species	**Isolation code**	**Accession number**
Leptosphaerulina trifolii	WAC 6693	AY831543.1
Ascochyta pisi	AP2	DQ386494.1
teleomorf: *Didymella lentis* anamorf: *Ascochyta lentis*	SAT AL	AY831546.1
Ascochyta fabae f. sp. *viciae* (= *Ascochyta fabae*)	AV11	DQ386498.1
teleomorf: *Didymella lentis* anamorf: *Ascochyta lentis*	AL1	DQ386493.1
teleomorf: *Didymella fabae* anamorf: *Ascochyta fabae*	AF1	DQ386492.1
Claviceps sorghi	?	AY960837.1
Claviceps sorghi	?	AY960836.1
Phoma pinodella	CBS 318.90	AY831542.1
Phoma pinodella	WAC 7978	AY831545.1

According to the phylogenetic tree based on *tef*1 sequences (Figure 3), the *Phoma* species are well separated from their closely related *Ascochyta* taxa.

As the identification of *Phoma* and *Ascochyta* genus based on morphological characteristics is often problematic, this new phylogenetic marker can be a useful tool for mycologists identifying an unknown species.

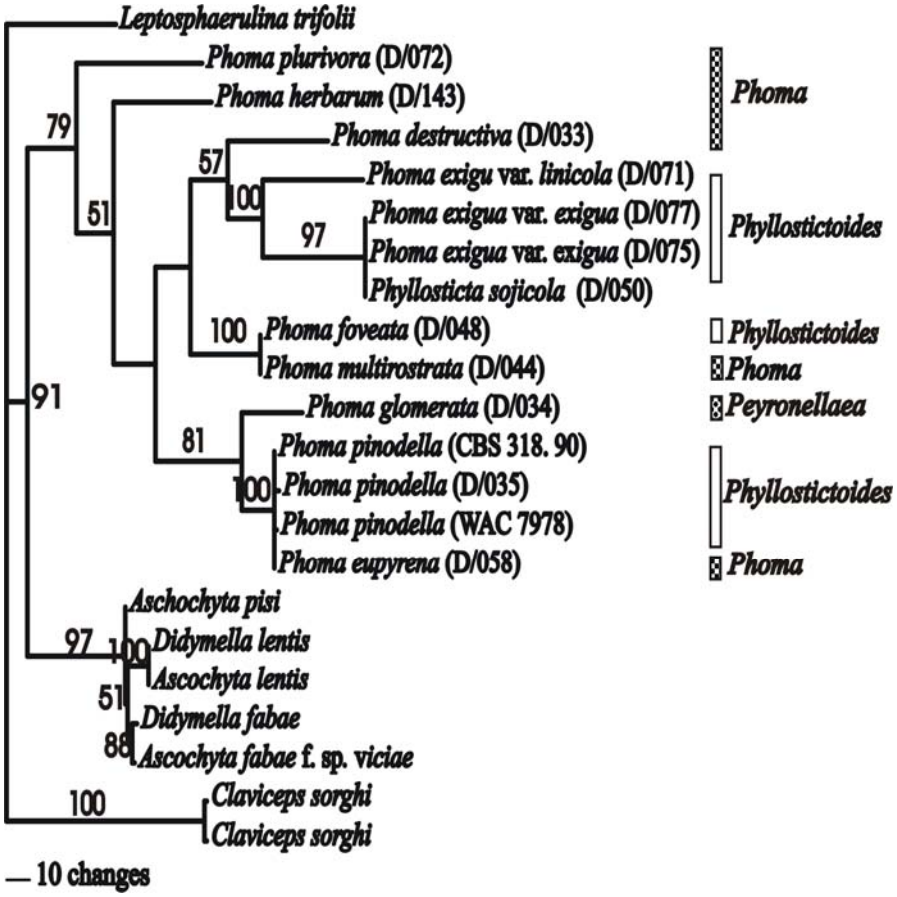

Figure 3. Phylogenetic relationships of *Phoma* strains inferred by the parsimony analysis of *tef1* sequences. The numbers above the lines represent the bootstrap (bootstrap=1000) values. The columns on the right side represent the *Phoma* sections based on morphological characterization

Most of *Phoma* species (*P. plurivora, P. herbarum, P. desrtuctiva, P. glomerata*) are well separated from the other tested *Phoma* species. Some *Phoma* species constitute clades but there are some species which can not be distinguished on the basis of *tef1* sequences (*Phoma pinodella* and *Phoma eupyrena* as well as *Phoma foveata* and *Phoma multirostrata*). The species represented by more than one isolate are classified in the same subgroup, which proves that the *tef1* sequences are well suited for delineating phylogenetic relationships within the *Phoma* genus.

The *Phyllosticta sojicola* associates with the *Phoma exigua* var. *exigua* subgroups which support the statement that the two species are identical (Kövics et al., 1999).

The phylogenetic tree based on *tef1* sequences does not support the traditional *Phoma* sections based on morphological characterization (Boerema et al., 2004).

On the basis of our investigation, carried out with *tef1* sequences all *Phoma* species form a well distinguishable group from the *Ascochyta* species, which proves the monophyletic origin of *Phoma* genus.

Up to now, phylogenetic analyses within *Phoma* genus have only been used for defining phylogenetic relationships among isolates within one species (Mendes et al., 2003 and Balmas et al., 2005).

Here we have used the translational elongation factor to resolve phylogenetic relationships within *Phoma* genus at higher taxonomic levels.

The present study has proved the *tef1* region to be phylogenetically useful tool for defining *Phoma* species but further investigations would be necessary to clarify whether the *tef1* gene sequence as phylogenetic molecular marker is well suited for the classification of *Phoma* species.

ITS sequences

In the PCR reaction 0.6kb fragment of the rDNA gene containing the internal transcribed spacer regions 1 and 2 and the 5.8S regions was amplified.

For phylogenetic analyses of ITS region we involved other *Phoma* and *Ascochyta* species as well as *Didymella* and *Leptosphaeria* species as outgroup, all were downloaded from GenBank maintained by the NCBI (Table 3).

The phylogenetic tree based on ITS sequences (Figure 4) is drawn by parsimony analyis.

The difference between the different *Phoma* and *Ascochyta*, *Leptosphaeria* and *Didymella* species was not significant, 23 sites were considered as informative for the parsimony analysis. Moreover only 3 clades were supported by the bootstrap analysis with more than 80% probability.

We can state from the parsimony tree that the *Phyllosticta sojicola* grouped with the *Phoma exigua*, as we were able to state from the analysis of *tef1* sequences. *Phoma foveata* and *Phoma multirostrata* also grouped together both in ITS and *tef1* analysis.

ITS sequences should analyzed with other methods (like MEGA or maximum likelihood), which may draw a much supported tree.

Table 3. Species involved in the phylogenetic analyses of ITS fragments

Species	**Isolation code**	**Accession number**
Phoma exigua var. *heteromorpha*	?	AY899262.1
Phoma exigua	CSL 20316964	AY550992.1
Phoma exigua var. *populi*	CBS 100167	AF268189.1
Phoma exigua var. *linicola*	CBS 113.28	AF268187.1
Phoma exigua	?	AY927784.1
Phoma herbarum	?	DQ132841.1
Phoma herbarum	ATCC 12569	AY293803.1
Phoma pinodella	VPRI 32177	DQ087402.1
Phoma pinodella	VPRI 32171	DQ087400.1
Phoma pinodella	CBS 318.90	AY831562.1
Phoma pinodella	WAC 7978	AY831556.1
Phoma glomerata	?	AF126816.1
Phoma glomerata	?	AY618248.1
Phoma glomerata	?	AY183371.1
Phoma eupyrena	Gr61	AJ890436.1
Leptosphaerulina trifolii	WAC 6693	AY831558.1
Ascochyta sp.	Georgia6	DQ383955.1
Ascochyta pisi	AP1	DQ383954.1
Ascochyta lentis	MU AL1	AY131201.1
Didymella lentis	AL1	DQ383953.1
Didymella fabae	AF1	DQ383952.1

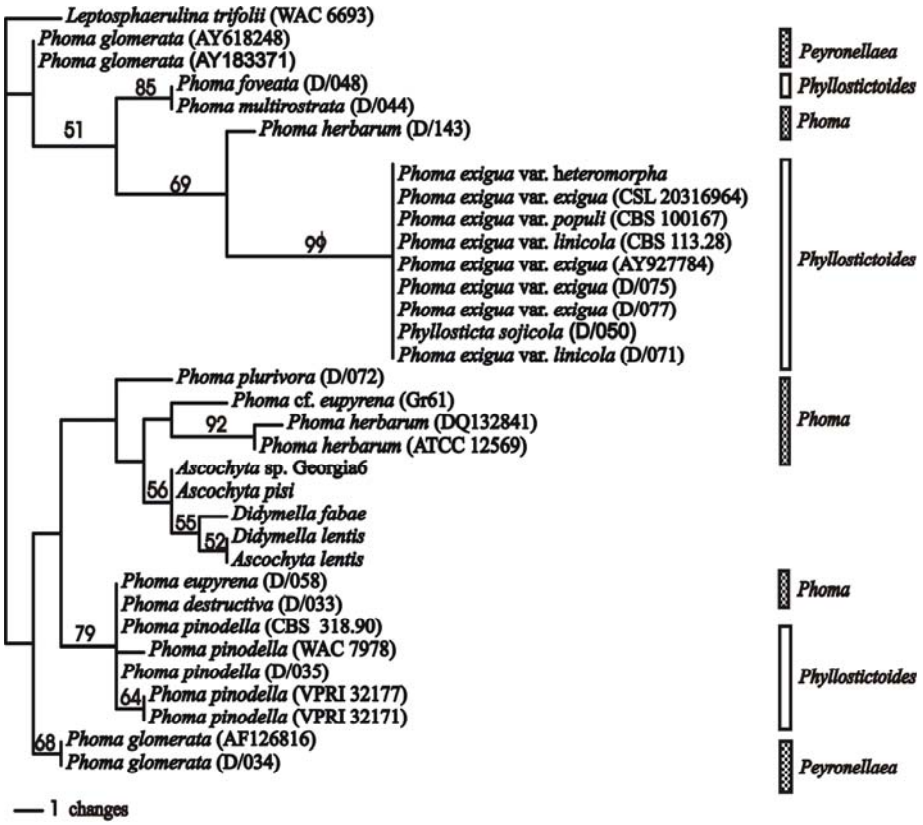

Figure 4. Phylogenetic relationships of *Phoma* strains inferred by the parsimony analysis of ITS sequences. The numbers above the lines represent the bootstrap (bootstrap=1000) values. The columns on the right side represent the *Phoma* section based on morphological characterization.

Discussion

tef1 sequences are well suitable for phylogenetic analysis of *Phoma* species similarly to other mycetous fungi (Druzhinina and Kubicek, 2005). The phylogenetic analysis of ITS sequences by parsimony method has not given a definite result. One possible reason for this can be that there were limited variable sites in ITS sequences. This can mean that the evolutionary distance by ITS sequences within *Phoma* species is too small to get well based consequences for the phylogenetic relationships of *Phoma* genus. So similarly to the *Trichoderma* genus we should involved other sequences in phylogenetic analysis like *tef1* or β-tubulin sequences.

Both sequence analyses confirmed that the *Phyllosticta sojicola* species is identical to the *Phoma exigua* var. *exigua* species as Kövics et al., 1999 claimed.

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STUDIES OF EVOLUTIONARY RELATIONSHIPS OF *PHOMA* SPECIES BASED ON PHYLOGENETIC MARKERS

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Summary

The cosmopolitan *Phoma* genus contains mainly phytopathogenic, opportunistic parasite, and saprophyte fungal species. Up to now the characterization of *Phoma* species and other taxa of *Phoma* has so far been determined on the basis of morphology on standardized media, and gene sequence analysis was only used as a confirmative or distinctive complement.

In this study we have tried to find molecular markers which can be used as phylogenetic markers in the molecular based classification in the *Phoma* genus.

We employed a part of the translation elongation factor 1 subunit alpha (EF-1α=tef1) containing both introns and exons and ITS region containing the internal transcribed spacer regions 1 and 2 and the 5.8S rDNA, as a potential genetic markers to infer phylogenetic relationships among different *Phoma* taxa. Twelve different *Phoma* species sequences were analysed together with the closely related *Ascochyta* ones. The constructed phylogenetic trees based on *tef1* and ITS sequences, do not support the traditional *Phoma* sections based on morphological characterization. However we have managed to distinct the *Phoma* strains and *Ascochyta* species comparing their *tef1* sequences by parsimony analysis. We have proved that a *tef1* can be a useful phylogenetic marker to resolve phylogenetic relationships at species level in *Phoma* genus.

Both parsimony sequence analyses confirmed that the *Phyllosticta sojicola* species is identical to the *Phoma exigua* var. *exigua* species as Kövics et al. (1999) claimed. However the evolutionary distance by ITS sequences within *Phoma* species is too small to get well based consequences for the phylogenetic relationships of *Phoma* genus.

Further investigations would be necessary to clarify whether the *tef1* and ITS sequences as phylogenetic molecular markers are well suited for the classification of *Phoma* species.

ENTOMOLOGICAL AND INTEGRATED PEST MANAGEMENT SESSION

ONE NEUTRAL MODEL IN SPECIES ABUNDANCE DISTRIBUTION OF ARTHROPODS IN AGRO ECOSYSTEMS

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The relative species abundances distribution (RSA) has been experimentally studied for different plant and animal communities, inspiring theoretical works and modeling efforts (Tokeshi, 1993, 1999; Harte et al., 1999; Harte 2000; Hubbell 2001; Sizling and Storch, 2004). Targeting neutral-like communities where the individuals from different species compete with each other solely for the limited amount of resources are especially important nowadays for verifying the applicability of neutral macroecology models (Dewdney, 2000; Moulliot et al., 2000; Hubbell 2001; Maguran and Henderson, 2003; Norris, 2003). Most of the experimental data on RSA are for such communities (trees in tropical forest, moths, birds etc.) (Dirks, 1937; Condit et al., 1996; Condit, 1998; Keitt and Stanley, 1998) and confirm the prediction of neutral-like theories (Hubbel and Foster, 1983; Price et al., 1995; McGill, 2003; Norris, 2003; Sauer et al., 2003; Sizling and Storch, 2004; Ravasz et al., 2005; Néda et al., 2005). For the considered experimental samples the shape of RSA suggests at a first glance a qualitatively different behavior. Statistical methods on insect populations have been used for a long time to predict population outbreaks, extinctions or even endemics-area relationships (Pielou, 1977; Luff and Eyre, 1988; Pueyo, 2006). Unfortunately the empirical correlative methods usually failed to give satisfactory predictions as the unexplained or immeasurable sources of variations (winter disappearance, hide larval stages or even the collection methods) were large and not likely to be predicted. Most of the methods have limited applications because they lack the flexibility to incorporate in a dynamic way much of the relevant biology of the organisms involved and the vagaries of population survival through time as influenced by weather. For ecological and conservation purposes, it is thus of primary interest to find statistical laws, that could yield additional information on the

whole community and which could be later correlated with parameters that give information about the dynamics of the system. Apart of it's macroecological importance, obtaining information on the shape of the RSA could be helpful for understanding universal or specific features of insect communities and would give also an important testing ground for complex models that aim to reproduce or predict the dynamics of such communities (Luff and Eyre 1988).

Materials and Methods

Experimental data: Barro Colorado Island (BCI) data set Smithsonian Tropical Research Institute - rainforest ecosystem studies.
Trees are sampled in a 50-ha region, the first census was completed in 1983, with recensuses occurring every 5 years since. All stems greater than 10 mm diameter were mapped (Figure 1.).

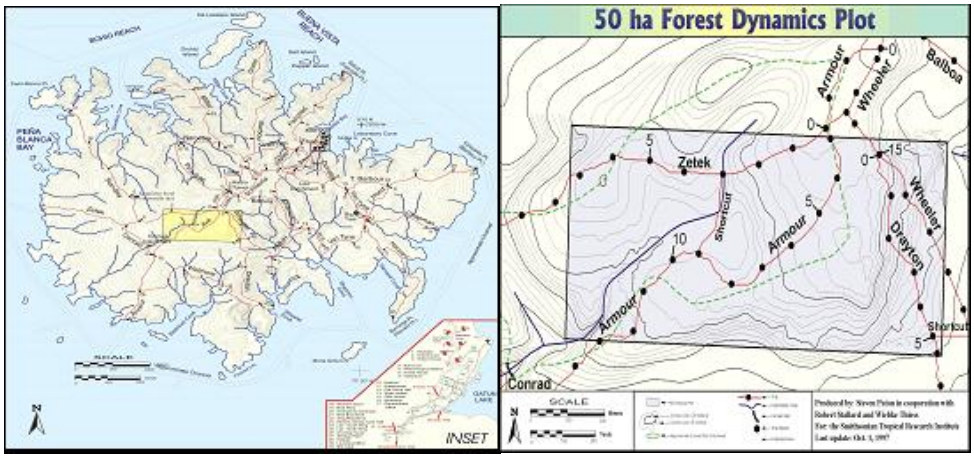

Figure 1. Barro Colorado Island experimental site.

Our data sets used were collected by us from agricultural environments (apple and pear orchards). Predacious insects were sampled from soil to canopy level in the periods 1998 and 2003 in Central-Europe - Hungary.
In apple and pear orchards for samples at the soil level, covered pitfall traps (300 cm^3 in size, 8 cm in diameter, half-filled with ethylene glycol 30% solution) were used between 1998 and 2003. Ten pitfall traps were placed in nine localities at 10-m intervals from each other. At the canopy level the insects were collected with conventional beating method. The investigated orchards were situated in three geographical regions and were surrounded by different habitats. These were agricultural lowland environments, regularly flooded area and woodland areas of medium height mountains. The soil compositions in the investigated orchards were sand, sandy-loam

and clay, respectively. In some orchards intensive treatments were used and the pest management was based on wide spectrum (mainly organophosphorus) insecticides (Ultracid 50 WP, Zolone 35 EC, Dimecron 50 WP). The other orchards were treated with integrated pest management methods (IPM) or were untreated, i.e. neither pesticides nor fertilizers were used for five years before we started our investigations. We have to emphasize here that our results suggested no significant differences in the species composition and activity abundances for the differently managed orchards. In these orchards the studied insect orders and families were rove beetles (*Coleoptera: Staphylinidae*) collected from soil and canopy level and ground beetles (*Coleoptera: Carabidae*) collected only from soil level.
The statistics of the species abundances were studied in different levels, time-periods and spatial resolution, grouping or not different families. The $\Psi(r)$ probability density characterizing the relative species abundances distribution (RSA) was computed by the classical method: counting the number N_k of species with sizes, r, between 2^k and 2^{k+1} ($k=1,2,3$ ….), i.e. constructing a histogram on intervals that are not of constant length, but are exponentially increasing. In this manner the large statistical fluctuations from the tail of the distribution function is greatly reduced. In order to obtain the rigorously defined probability density, $\Psi(r)$, one must divide N_k with the exponentially increasing size of the interval, $\Delta r=2^k$, and by the total number of species, N: $\Psi(r)=N_k/(N.2^x)$. Constructing the distribution functions in this manner one will obtain a monotonically decreasing function, which for a good statistics can be usually well approximated with a power-law that presents and exponential cutoff. Plotting it on a log-log scale one obtains thus the characteristic tilted **J-shape** curve. The initial slope of the $\Psi(r)$ curves (the power-law behavior) will characterize the scale-invariant nature of the RSA.

Results

Our model is a complex, spatially extended model combine the essential parameters of mean-field type approaches (birth, migration, death) and the spatiality of Potts-type models. The square lattice definition - each lattice site can be occupied by many individuals belonging to different species - consequently a lattice site corresponds to an area in which the spatiality is neglected.
Realistically the total number of individuals on a lattice site isn't fixed, it can fluctuate around a given N_0 value that characterizes the limited amount of available resources in a given territory. The death rate (**d**) depends on the

instantaneous number of individuals (**N**) in each lattice site according to the following relation: $d = d(N) = 1 - \frac{1 - d_0}{e^{\frac{N - N_0}{\alpha}} + 1}$

If N>N0, the death probability increases (if an area become over-populated, the birth/death rate decreases). d_0 is the death rate when N=0. If **N** tends to ∞ the death rate tends to 1. The α parameter controls the steepness of the curve: the smaller the value of α, the steeper the curve (Figure 2).

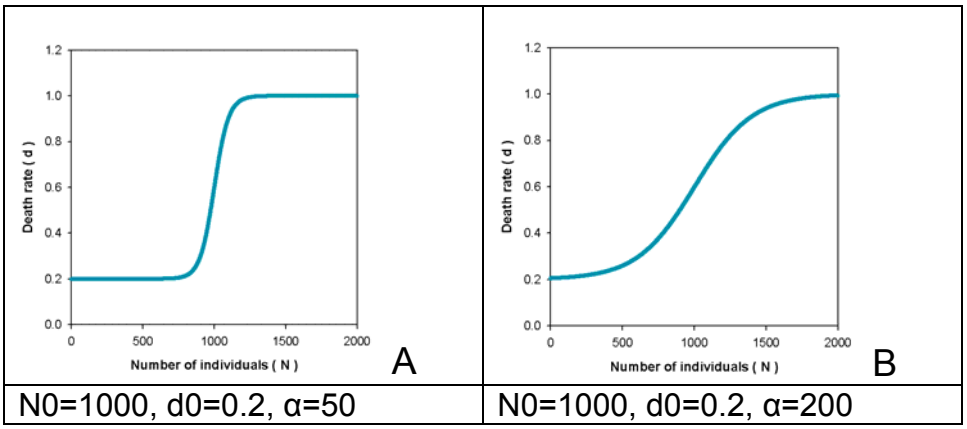

Figure 2. The graphs of the death-rate function in case of two different (N0, d0, α) combinations.

Individuals in the system can give birth to individuals belonging to the same or to a new species (mutation) with **b** probability and **m** respectively.
The spatiality of the model implies that the individuals can migrate to one of the neighboring sites (cells). This migration is characterized by the ***q*** migration rate.
With ***w*** probability an individual from a randomly chosen species can be assigned to a randomly chosen lattice site – immigration of individuals from the metacommunity. While in case of mutation a new species shows up, in case of immigration the species of the immigrant individual is randomly selected from the defined set of species.

The dynamics of the model

1. the size of the square lattice
2. the number of species in the metacommunity
3. the parameters of the model: d0, α, b, m, q and w
4. the initial number of individuals on the lattice: Z
5. Initialization of the system: Z individuals from randomly chosen species are assigned to randomly chosen lattice sites

6. Starting the dynamics if the system: In each simulation step with the initially fixed probabilities each individual can double itself, give birth to an individual belonging to a new species, die or migrate to one of the neighboring sites. With initially fixed probability one individual from randomly chosen species can enter into the system to a random position (immigration)
7. The dynamics goes on until the dynamical equilibrium sets in.

Simulation is done with the kinetic Monte Carlo method (BKL method).
The spatial distribution of the most abundant species was presented in Figure 3, where the experimental dates (BCI) and the simulation dates are compared.

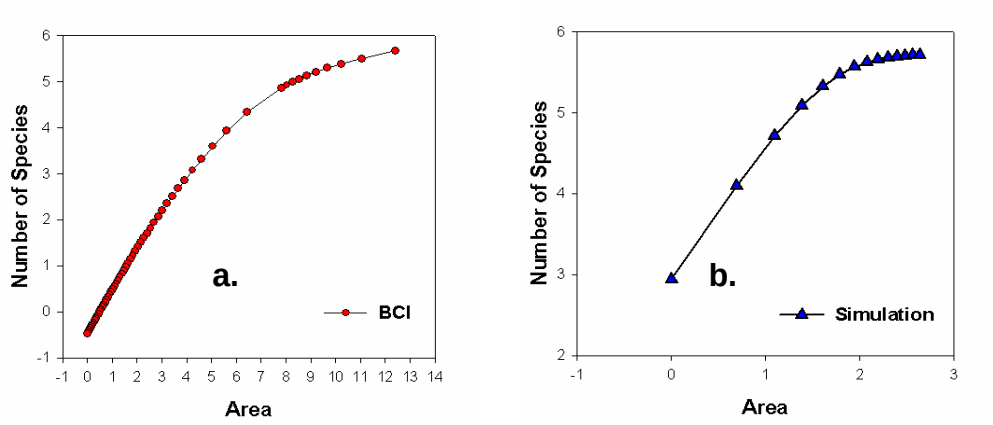

Figure 3. Spatial distribution of the most abundant species, **a.** experimental (BCI), **b.** simulation, simulation parameters: lattice size=15, initial number of species=400, initial number of individuals=5000 b=0.3, m=10-5, q=5*10-4, w=10-5, d0=0.1, N0=1500, α=50

Application

First we present the results obtained for the RSA in apple and pear orchards focusing on rove beetles (*Coleoptera: Staphylinidae*). Our statistics here is based on total of 7310 individuals collected in nine different locations during a five year period, with ten pitfall traps in each location.
On Figures 4a through 4c we consecutively consider larger and larger samples including more and more locations and sampling time. On Figure 4a we have plotted on a log-log scale the RSA obtained in one year (2000) for three different locations, considering for each location the rove beetles collected in all pitfall traps (1289 individuals from 61 species). Studying the RSA separately for each trap is not relevant, since the number of collected individuals in one year and one trap is too small for a reasonable statistics. Although the statistics for one year and one location is not impressive either

(in medium we have 430 individuals) the probability density suggest a power-law behavior, with an exponent between -1.44 and -1.9. For guiding the eye we have plotted the power-law with -1 exponent that usually characterizes the RSA for neutral-like communities. On Figure 4b the RSA is plotted for the same three locations but considering now the individuals collected during the whole study period, i.e from 1998 to 2002 (3191 individuals from 162 species). The shape of the RSA suggests again a scale-free (power-law) behavior on an interval larger than two orders of magnitude. The calculated scaling exponents are between -1.48 and -1.63. Finally, on Figure 4c we have plotted the RSA obtained from the whole data set, considering all the traps from all locations and for all the study years (7210 individuals from 250 species). The result suggests again a power-law nature, with a scaling exponent -1.51.

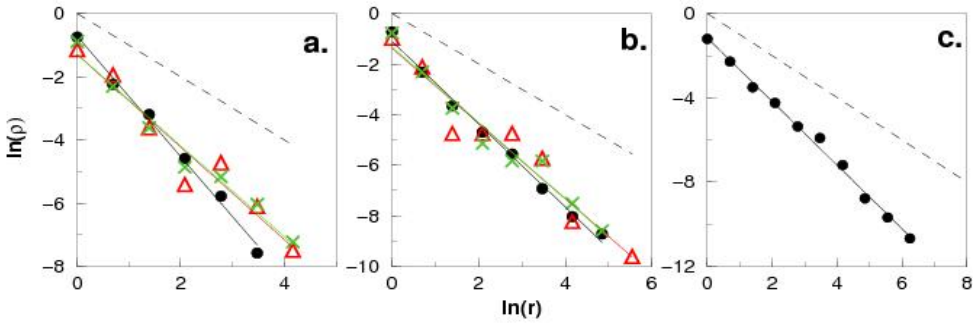

Figure 4. RSA for rove beetles *(Coleoptera: Staphylinidae)* sampled in apple and pear orchards. Figure **a** presents results for a one year sampling (2000) in three different location in Hungary (East Europe), using ten pitfall traps in each location. Figure **b** presents results for the same three locations, but considering a five year period sampling. Figure **c** presents the RSA for the whole sampled community (nine locations during a five year sampling period). On all graphs the dashed line indicates the power-law behavior with scaling exponent -1.

In the same apple and pear orchards we have studied also the RSA for ground beetles (*Coleoptera: Carabidae*). On figs 5a-5c the same type of graphs are plotted as for the rove beetles in fig 4a-4c. The results are also pretty similar, although for this insect family we had a much better statistics, based on a total number of 25580 individuals from 172 species. This is clearly visible on fig 5c, where a scaling law is visible for the shape of the RSA extending on almost four orders of magnitude! The scaling exponent for the whole sampled community is -1.35, somewhat smaller than the value obtained for rove beetles.

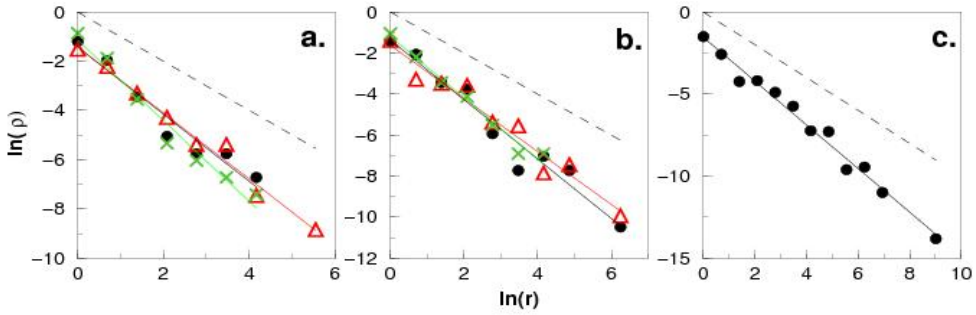

Figure 5. RSA for ground beetles *(Coleoptera: Carabidae*) sampled in the same apple and pear orchards. Figure **a** presents results for a one year sampling (2000) in the same three different location in Hungary, East Europe (the exponents of the power-law fit are between -1.34 to -1.64). Figure **b** presents results for the same three locations, but considering a five year period sampling (the exponents of the power-law fit are between -1.25 and -1.49). Figure **c** presents the RSA for the whole sampled community (nine different locations during a five year sampling period). On all graphs the dashed line indicates the power-law behavior with exponent -1.

Considering now together the two insect orders (32790 individuals of rove and ground beetles from 422 species) and computing the RSA for the whole sampled community (five year sampling period, nine different locations) we already get a curve that resembles the tilted J shape observed in neutral-like communities (Figure 6). The exponent for the initial part of the RSA, -1.3, is however different from the usually observed -1 value.

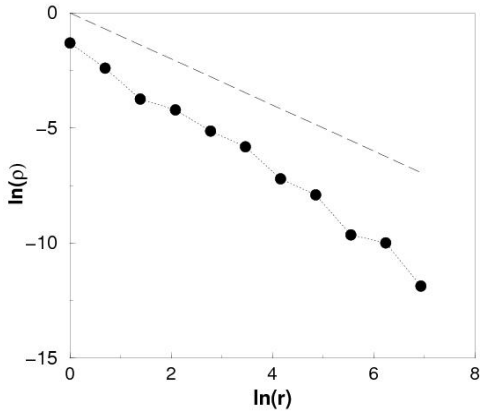

Figure 6. RSA considering together rove and ground beetles *(Coleoptera: Staphylinidae* and *Carabidae,* respectively), computed from a five year sampling period in nine different apple or pear orchards. The dashed line indicates the power-law behavior with exponent -1.

Preliminary results are encouraging and suggest that our approach is able to describe all major statistical aspects of neutral systems.
The model has to be investigated for various parameters and the results of the simulations have to be compared with other experimental databases.
Focusing on a specific insect family most of our data suggests a power-law nature for the RSA curve. From the results on ground beetles and rove beetles one can observe that the scaling can be valid on abundance intervals up to three orders of magnitude, which is quite impressive! Combining results for more years, more localities or traps does not destroy the apparent power-law nature of the RSA, although the scaling exponent can change in a broad interval ranging from -1.9 to -1.3. It is however too soon for concluding that for a given insect family the RSA has a power-law nature and sampling with even better statistics should be done. We believe that most of the insect species and many of the specimens are invisible for our sampling methods, especially the larval stage of many species could not be sampled as the adults and we have studied thus only the "top of the iceberg". This incomplete sampling could be also responsible for the fact that the scaling observed in the rare species limit does not show the 1/r – type behavior (-1 scaling exponent) observed for other communities (tropical trees, breeding birds).
The computer code is appropriate for pedagogical purposes, the software can be found in the following site:
URL: htp:/atom.ubcluj.ro/□aderzsi/neutral.html

Acknowledgments

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ONE NEUTRAL MODEL IN SPECIES ABUNDANCE DISTRIBUTION OF ARTHROPODS IN AGRO ECOSYSTEMS

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Summary

In this work we present and discuss results for RSA on insect's families, which are not necessarily neutral. The relative species abundances (RSA) are investigated for two insect families (rove beetles – *Coleoptera: Staphylinidae*, and ground beetles – *Coleoptera: Carabidae*).
For a given insect group the results obtained for the shape of RSA calculated from the rigorously defined probability density suggests a nontrivial scaling.
Studying the finite-size effects, we argued that accepting the form $f_0(x)$ for SAD, one can derive also an interesting scaling relation between the size of the most abundant species (N_s), the total number of individuals (N_T) and the number of detected species (S_T) in the considered habitat: $F(N_T,N_S,S_T)=S_T N_S/[N_T(\ln(N_S)-1)]=2$.
The observed trend is qualitatively different from the shape of the RSA computed from previous studies in neutral-like communities (tropical trees or breeding birds).
We argue however that the apparently different trend is not necessarily a real effect and could well be a consequence of the incomplete sampling. For a clear conclusion sampling with even better statistics should be performed.

ACTUAL PROBLEMS IN PLANT PROTECTION OF PEAR ORCHARDS

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So far, the application of the insecticides and fungicides has been determined by the requirement of the protection against the pear scab, pear psylla, codling moth and summer fruit tortrix moth. Recently, changes had to be made in the selection of fungicides and insecticides because resistances have been developed in a few years in the case of pear scab (*Venturia pyrina*) against fungicides and pear psylla (*Cacopsylla pyri*) against the insecticides.

The appearance of fire blight (*Erwinia amylovora*) and pear phytoplasma, as well as the withdrawal of the permission of numerous pesticides required new approaches in the plant protection in the pear orchards.

In order to reduce the spread of the fire blight the application of the fungicides containing copper active ingredient had to be increased, and the systematical removal of the dead branches and twigs was required. To prevent the further infection of the pear phytoplasma it is necessary to keep continuously at low level the density of pear psylla population.

The use of the dinitro-orto-cresol insecticides provided an effective protection against the pear psylla in early spring, however the permission of their use was withdrawn. According to our experiences the paraffin oil preparations could provide suitable protection against this pest, in this time. It is possible to keep the population density of *C. pyri* in the vegetation period at a low level by the repeated application of the paraffin oil preparations, the IGR insecticides (diflubenzuron, fenoxicarb), but first of all, by the use of Vermitec (abamectin).

Because of the consecutive ripening time of pear cultivars, the application of the numerous insecticides, recommended for the integrated pest management is not possible. Therefore, the prevention of damages of codling moth, summer fruit tortrix moth and pear psylla needs particular attention on the base of continuous observations.

THE INFLUENCE OF THE ADJACENT VEGETATION PATCHES ON DIVERSITY AND ABUNDANCE OF GREEN LACEWINGS ASSOCIATED TO THE OLIVE GROVES IN SOUTH SPAIN. IMPLICATIONS IN THE NATURAL CONTROL OF THE OLIVE MOTH, *PRAYS OLEAE* (LEP: YPONOMEUTIDAE)

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The olive moth, *Prays oleae* (Bernard) (Lep., Yponomeutidae) is responsible of reductions in crop production of olive trees up to 60%. Amongst the natural enemies of the olive moth, predation carried out by green lacewings larvae (Chrysopidae) is frequently reported. They feed mostly on *P. oleae* eggs, reaching usually very high levels of predation – greater than 90% – which revealed that Chrysopidae are essential agents in the modern olive culture. In the investigations carried out on the olive moth, a several lacewing species have been observed, and the common green lacewing, *Chrysoperla carnea* s.l. was dominant.

However, due to the trouble in the taxonomic status of *Chrysoperla carnea*-complex, recent investigations, directed to the knowledgede of the cryptic species present during the oviposition period of *Prays oleae* in south Spain groves, showed that *Chrysoperla agilis* was the most abundant of the *carnea*-group.

Preliminary studies suggested the existence of greater populations of *Chrysoperla carnea* s.l. during the early spring in natural oak forest vegetation bordering the olive groves. Are these patches of vegetation important reservoirs to maintain lacewing populations? In such case, which species of the *carnea*-group are mainly involved? Another important objective of this study is to evaluate their role in the natural control of *Prays oleae* and to compare it with a similar grove under different ecological conditions: bordered by coniferous forests.

The four species of the *carnea* group – *Chrysoperla agilis, Chrysoperla lucasina, Chrysoperla carnea* sensu stricto and *Chrysoperla affinis* - were collected in the olive grove bordered by oaks, whereas only *Ch. agilis* and *Ch. lucasina* were observed in the olive grove close to the pine forest (*Pinus halepensis*), where not lacewings were collected. In contrast, in the oak forest *Chrysoperla agilis* was collected even before the oviposition period

of *Prays oleae*, and it was also the dominant species in both olive groves during the summertime (>75% of the total ind. caught).

The greatest diversity of lacewings was observed in the passing area between grove and oak forest.

In relation to the big differences in diversity and relative abundance, signifficant differences were also found in egg predation by lacewing larvae betwen the two groves, as well as in the final level of infestation by *Prays oleae*. In addition, relevant agronomical implications of these results will be here discussed.

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TRIALS WITH OVERWINTERING CHAMBERS AS CONSERVATION TOOLS FOR COMMON GREEN LACEWINGS IN HUNGARY

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Under natural conditions the adults of common green lacewings (*Chrysoperla carnea* s.l. (Stephens, 1836)) overwinter among leaf litter, in rolled dry leaves, ivy tufts and unheated parts of buildings (Thierry et al., 1994) where their surviving can be hazardous and mortality is high (Şengonca and Frings, 1987). Lacewing overwintering chambers placed in the field and orchard may be colonized by adult common green lacewings (Şengonca and Frings, 1987, 1989; McEwen et al.,1999). These boxes can augment the natural lacewing population by saving the hibernating adults from the winter coldness and precipitation as well as the chambers - kept at a dry and protected place during winter - placed in the field in the spring may result in the earlier occurrence of adults and eggs (Şengonca and Henze, 1992). The use of these hibernating shelters may increase the density of natural populations of *Ch. carnea* s.l. and parallel enhances their biological control performance. It is worth mentioning that the taxonomic status of *Ch. carnea* has been changing, and instead of a polymorphic single species, a complex of sibling or cryptic species, the *Chrysoperla carnea* complex or *carnea*-group (Thierry et al., 1992; Thierry et al.,1998; Henry et al., 2001) should be now considered whose members` systematic status is not known enough (Tauber et al., 2000, Henry et al., 2001). According to many-sided investigations we can distinguish in mainland Europe three cryptic species: 1) *Ch. affinis* (Stephens, 1836) former *Ch. kolthoffi* (Thierry et al., 1998); 2) *Chrysoperla lucasina* (Lacroix, 1912) (Henry et al., 2001) and 3) *Chrysoperla carnea* sensu stricto (Thierry et al., 1998). The aim of the following experiments is to prove the efficiency of the chambers in Hungary and to study the overwintering behaviour of the sibling species of *Ch. carnea* complex.

Materials and Methods

The wooden boxes (15 cm x 15 cm x 15 cm) used in the experiment were made in Great-Britain according to the design of McEwen (1998). All chambers were tightly packed with straw. The front and bottom side of the boxes had louvred slats, three cm apart, through which lacewings can enter. The boxes were placed with the louvred side facing away from the

prevailing winds, however, local topography and changeable wind directions might influence results. Six chambers were placed in the field in Hungary in August or September, two in a fruit orchard in Gödöllő about 25 km from Budapest, and four at Debrecen in the north-east of the country. Chambers at the Gödöllő site were placed in an orchard in the garden suburb of the town. The orchard (about 1000 m^2) is surrounded by other orchards and gardens. In the vicinity of the chosen orchard there is an extended uncultivated semi-natural area covered by deciduous trees and shrubs and a deciduous forest comprising oaks, maples and linden trees. The orchard itself contains a variety of fruit trees and bushes. The chambers were fixed on the top of fence posts (at a height of 150 – 160 cm), the distance between chambers being about 30 m. The four chambers at Debrecen were placed in the experimental area of the Agricultural Centre situated on the outskirts of the town. This area (about 5 ha) is bordered by the University campus and fields and is planted with a variety of fruit trees and other crops. Chambers were mounted on wooden stakes at a height of 150 – 160 cm. Table 1 shows mounting, collecting and emptying data.

Table 1. Dates of mounting, collecting and emptying of overwintering boxes

Site and date	No. of boxes	Mounting	Collecting	Emptying
Debrecen 1997	4	30 08	17 11	17 11
Gödöllő 1997	2	28 08	15 11	15 11
Debrecen 1998	4	02 09	17 11	17 11
Gödöllő 1998	2	04 09	24 11	24 11
Debrecen 1999	4	14 09	24 11	24 11
Gödöllő 1999	2	19 09	04 12	16 12
Debrecen 2000	4	27 08	21 11	2001 03 04
Debrecen 2004	4	14 09	11 11	2005 22 03
Debrecen 2005	4	21 09	17 11	2006 28 03

Results and Discussion

The number of lacewings that colonized the hibernation boxes at the two sites are listed in Tables 2-7. Besides the lacewings other insects (coccinellids (*Adalia bipunctata*), other small coleoptera, bugs (Miridae, Anthocoridae), moths, flies (Syrphidae), wasps (*Polistes gallicus, Paravespula vulgaris*), mosquitoes (*Culex* sp.) and spiders were found. These data correspond to the former studies (McEwen et al., 1999; Weihrauch, 2004).

Table 2. Common green lacewings colonizing overwintering chambers (1997) (in brackets number of females)

Site	*Ch. affinis*	*Ch. carnea* s.str.	*Ch. carnea* s.l.	Total
Debrecen	2 (1)	-	-	2 (1)
	31 (14)	1 (-)	2 (-)	34 (14)
	-	-	-	-
	59 (22)	1 (-)	3 (-)	63 (22)
Total	92 (37)	1 (-)	5 (-)	99 (37)
Gödöllő	1 (-)	-	-	1 (-)
	-	-	-	-
Total	1 (-)	-	-	1 (-)

Table 3. Common green lacewings colonizing overwintering chambers (1998) (in brackets number of females)

Site	*Ch. affinis*	*Ch. carnea* s.str.	*Ch. carnea* s.l.	Total
Debrecen	4 (3)	-	-	4 (3)
	9 (8)	-	-	9 (8)
	3 (2)	-	-	3 (2)
	2 (2)	-	-	2 (2)
Total	18 (17)	-	-	18 (17)
Gödöllő	-	-	-	-
	-	-	-	-
Total	-	-	-	-

Table 4. Common green lacewings colonizing overwintering chambers (1999) (in brackets number of females)

Site	*Ch. affinis*	*Ch. carnea* s.str.	*Ch. carnea* s.l.	Total
Debrecen	4 (3)	-	-	4 (3)
	2 (1)	-	-	2 (1)
	-	-	-	-
	23 (10)	-	-	23 (10)
Total	29 (14)	-	-	29 (14)
Gödöllő	-	-	-	-
	-	-	-	-
Total	-	-	-	-

Table 5. Common green lacewings colonizing overwintering chambers (2000) (in brackets number of females)

Site	*Ch. affinis*	*Ch. carnea* s.str.	*Ch. carnea* s.l.	Total
Debrecen	-	-	-	-
	4 (3)	-	-	4 (3)
	-	-	-	-
	-	-	-	-
Total	4 (3)	-	-	4 (3)

Table 6. Common green lacewings colonizing overwintering chambers (2004) (in brackets number of females)

Site	*Ch. affinis*	*Ch. carnea* s.str.	*Ch. carnea* s.l.	Total
Debrecen	21 (6)	-	-	21 (6)
	13 (4)	-	-	13 (4)
	1 (-)	-	-	1 (-)
	9 (4)	-	-	9 (4)
Total	44 (14)	-	-	44 (14)

Table 7. Common green lacewings colonizing overwintering chambers (2005) (in brackets number of females)

Site	*Ch. affinis*	*Ch. carnea* s.str.	*Ch. carnea* s.l.	Total
Debrecen	3 (3)	-	-	3 (3)
	3 (2)	-	-	3 (2)
	4 (3)	-	-	4 (3)
	-	-	-	-
Total	10 (8)	-	-	10 (8)

The number of individuals observed in the chambers was very variable from year to year. The single constant result was that the number of lacewings found in Gödöllő was minimal or nil. This could be due to the somehow covered form of the habitat which could not allowed the lacewings to discover the boxes. As to some of the Debrecen observations reasons for the poor results are not really clear but they can be explained mainly by the local environmental conditions (changeable wind direction, wet weather).
For example the weather in 1998 was very wet in both Debrecen and Gödöllő, some boxes got soaked and the straw became wet inside the box.

Table 8. Common green lacewings colonizing overwintering chambers in the period examined (n = number of overwintering chambers, in brackets percentage of females)

Site and year	n	Common green lacewings		
		Mean	Min	Max
Debrecen 1997	4	24.75 (37.4)	0	63
Gödöllő 1997	2	0.5	0	1
Debrecen 1998	4	4.5 (94.4)	2	9
Gödöllő 1998	2	-		
Debrecen 1999	4	7.25 (48.3)	0	23
Gödöllő 1999	2	-		
Debrecen 2000	4	1 (75)	0	4
Debrecen 2004	4	11 (31.8)	1	21
Debrecen 2005	4	2.5 (80)	0	4

Table 9. Number of sibling species of *Ch. carnea* complex colonizing overwintering chambers in the period examined (n = number of chambers, in brackets percentage of dominance)

Site and year	n	Sibling species		
		Ch. affinis	*Ch. carnea* s.str.	*Ch. carnea* s.l.
Debrecen 1997	4	92 (93.9)	1 (1.0)	5 (5.1)
Gödöllő 1997	2	1 (100)	-	-
Debrecen 1998	4	18 (100)	-	-
Gödöllő 1998	2	-	-	-
Debrecen 1999	4	29 (100)	-	-
Gödöllő 1999	2	-	-	-
Debrecen 2000	4	4 (100)	-	-
Debrecen 2004	4	44 (100)	-	-
Debrecen 2005	4	10 (100)	-	-
Total		198 (97.5)	1 (0.5)	5 (2.5)

Interestingly, the poorest results were observed in 2000 when the autumn and winter was not exceptionally wet. Until 1999 the boxes were emptied in November or December immediately after demounting them so the protecting efficiency of the boxes could not be assessed. However, in case of the three last years, the boxes were placed in an unheated wooden building and emptied only in the spring. These treatments showed a good

thermo-protecting effect of hibernating boxes. It should be stressed that in 2005 the winter was extremely cold, but the lacewings survived. In terms of the number of attracted lacewings in other countries, the Hungarian results are not unexpected. The lacewing average abundance per chamber differed also in various countries. In France 13.8 (Thierry et al., 2002), Sweden 56.8, Hungary 24.7, Finland 2.5, England 0.13-14 (McEwen et al., 1999) individuals were collected averagely. The biggest average abundance was harvested in Germany, 254 (Weihrauch, 2004) and 447 (Frings and Şengonca, 1988) individuals. The big differences may be due to local biodiversity, numbers of lacewings present, as well as local topography, changeable wind directions and meteorological conditions but the reasons for the considerable variations are not really apparent. In case of the first German example the forest mimicking support structure of hops garden could be attractive for the lacewings looking for overwintering sites (Weihrauch, 2004). In Great-Britain (Wales) the poorest results (0.13 individual as a mean of 30 chambers, and with a maximal value of one) were observed in an exceptionally wet autumn and winter (McEwen et al., 1999).

Regarding the sibling species composition, *Ch. affinis* was the most abundant species (97.5%) which was followed by *Ch. carnea* s.str. (0.5%). Vis-à-vis the third cryptic species, *Ch. lucasina,* no specimen was uncovered in the chambers. This can be explained with the rarity of *Ch. lucasina* and also that this species prefers natural hibernation places (Thierry et al., 1994). 2.5% of the individuals could not be identified because of the considerable variability of their characteristic traits (Table 9). The proportion of the species attracted was enormously similar to that of found in other countries. 98% (1297 ind.) *Ch. carnea* (synonym for *Ch. affinis*), 1.7% (23 ind.) *Chrysoperla pallida* (synonym for *Ch. carnea* s.str.) and 0.23% (3 ind.) *Ch. lucasina* were observed in overwintering chambers in Germany (Bavaria) (Weihrauch, 2004). The data in France (Loire valley) are almost the same: 94.9% (111 ind.) *Ch. kolthoffi* (former name of *Ch. affinis*), 4.3% (5 ind.) *Ch. carnea* s.str., 0.8% (1 ind.) *Ch. lucasina* (Thierry et al., 2002). When summarising the results with hibernation chambers from 2002-2004 in Germany the species composition changes a bit: *Ch. carnea* (synonym for *Ch. affinis*) amounted 89.5% (3183 ind.), *Ch. pallida* (synonym for *Ch. carnea* s.str.) 10.1% (359 ind.) and *Ch. lucasina* 0.4 (14 ind.) (Weihrauch, 2005).The French authors investigated the preferred hibernation sites of the sibling species and found that only *Ch. affinis* favours artificial shelters (unheated buildings), the other taxa prefer rolled dry leaves (*Ch. carnea* s.str.) and ivy tufts (*Ch. lucasina*) (Thierry et al., 1994). The German and Hungarian data concerning the proportion of specimens attracted in overwintering boxes correspond to the proportion of the assessed natural population (Table 10).

Table 10. Comparison of assessed natural populations of *Ch carnea* complex in Hungary and Germany

Country and year	*Ch. affinis*	*Ch. carnea* s.str.	*Ch. lucasina*	Total ind.	References
Hungary 1996, 1998, 1999	83.0	12.1	2.5	2010	Bozsik, 2000 (unpublished)
Germany 2001	84.7	9.5	2.0	1279	Gruppe, 2002

Attractive power of overwintering chambers and/or conditions influencing their attractiveness and the wandering of lacewings are not known enough. Local biodiversity, numbers of lacewings present, as well as local topography (coverage of chambers), changeable wind directions and meteorological conditions can be the most important reasons for the variations. In conclusion, thorough site selection respecting topography, wind direction and lacewing abundance, use of wind-adjusting chambers and attractants can contribute to the improvement of these devices that is further work is required to learn more about lacewings, their ecology and behaviour.

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TRIALS WITH OVERWINTERING CHAMBERS AS CONSERVATION TOOLS FOR COMMON GREEN LACEWINGS IN HUNGARY

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Summary

Under natural conditions the common green lacewings (C*hrysoperla carnea* s.l.) overwinter among leaf litter, in rolled dry leaves, ivy tufts and unheated parts of buildings where their surviving can be more hazardous. Their surviving rates may be increased by overwintering chambers placed in the field. The chambers can augment the natural lacewing population by saving the hibernating adults from the winter coldness and precipitation as well as the chambers placed in the field in the spring may result in the earlier occurrence of adults and eggs. The boxes placed in two different regions during six years protected efficiently the lacewings in winter, however, results must have been influenced strongly by local topography and changeable wind directions. Presence of *Chrysoperla affinis* individuals (97.5%) predominated in the boxes, but a few *Chrysoperla carnea* s.str. (0.5%) were found, too. Regarding the third cryptic species, *Chrysoperla lucasina,* any specimen was not uncovered in the chambers. 2.5% of the individuals could not be identified because of the considerable variability of characteristic traits.

LACEWINGS' OCCURRENCE IN SOME HUNGARIAN HEDGEROWS AND FIELD EDGES

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The relevant literature shows that the more heterogeneous vegetation bordering or surrounding cultivated areas supply sites for reproduction, overwintering and feeding for beneficial organisms, serving them as refugia, from which they can colonize/recolonize the cultivated areas (Van Emden and Williams, 1974; Horn, 1981). The most important bordering semi-natural vegetational formations are in Europe the hedges or hedgerows. Although the importance of lacewings as natural enemies of serious pests is entirely high, only a few studies have been carried out concerning their presence in hedges and influence of vegetation on these insects (Horn, 1981; Pantaleoni and Sproccati, 1987). In addition, though there are a lot of information on hedges and their relationship with natural enemies in northern or western European countries, in states with post-communist history these reports are rarities. For that reason, the objective of this study was to describe and compare the cenological characteristics of lacewing assemblages investigated in four hedges with different vegetational diversity.

Materials and Methods

Four different areas were studied in the north of Hungary from late April until late August in 1990-92. Two of the areas were situated in Gödöllő and the others in Kartal. The town of Gödöllő is located on the western slope of the watershed line of Cserhát mountain range and on both side of Rákos brook. It lies about 30 km from Budapest. The town has to be considered as the town of parks having in its center a huge park system and being rich in deciduous and coniferous trees.

The first area (Gödöllő1) was an about five km long coherent uncultivated territory (semi-natural wooded hedge in Gödöllő) grown over with diverse herbaceous, shrubby and woody vegetation, but the woody vegetation predominated (*Acer campestris, Acer negundo, Acer pseudo-platanus, Eleagnus angustifolius, Juglans regia, Prunus domestica, Prunus spinosa, Ulmus campestris, Tilia cordata, Crataegus monogyna, Evonymus europeus, Rosa canina, Sambucus nigra, Quercus* sp.*, Populus* sp.). The site was surrounded from the north and west by the railway line and a high way. The garden-city of Gödöllő was situated on the opposite side of high way. In the south and east the area was bordered by a meadow and cereal fields.

Beyond the fields ca. 1.5 km south-east of the first sampling site lay a deciduous forest.

The second area (Gödöllő2) was an about 400 m long hedgerow in the agricultural area of Gödöllő, which contained robinia trees (*Robinia pseudo-acacia*) and harboured a variegated weed flora. The acacia stand was very dense. The site was surrounded by cultivated field crops (potato, alfalfa, sunflower, barley). It was located ca. 6 km south-west from the first area.

The third site (Kartal1), an about 300 m long field border north from Kartal (small village situated 15 km from Gödöllő), composed of singly and thinly grown trees and bushes (*R. pseudo-acacia, Populus* sp.*, R. canina, S. nigra*) and some weedy plants.

The fourth area (Kartal2), another, a 500 m long field border near Kartal1, was characterized by sparsely grown trees (fals-acacias: *R. pseudo-acacia*) with less considerable herbaceous vegetation, and it was surrounded by cereal fields.

The localities were sampled weekly by sweeping net with 34 cm diameter and 100 strokes were made at shrub and crown level. No insecticide treatments were carried out during the study period.

When characterizing the chrysopid assemblages of the 4 different areas, the community structure parameters and indices like species richness, number of specimens, the Shannon-Weaver's diversity index ($H = - \Sigma p_i \log p_i$, where p_i is the proportion of i-th species in the sample; Southwood, 1984), and equitability were used. Equitability was calculated according to the formula: $e = H/\ln S$, where H was the value of diversity and S the number of species. The values of structure parameters mentioned above were analyzed by using two tailed t-test and regression analysis. The Rényi diversity as scale dependent diversity characterization ($H_a = [\log \Sigma p_i^a]l(1-a)$ was also calculated. Similarity values were processed by hierarchical cluster analysis using the group average clustering strategy (UPGMA) and Matusita distance. Also principal component analysis (PCA, standardized, centered) was calculated. In case of the vegetation only species presence and absence (binary) data were available, consequently their similarity by hierarchical cluster analysis (group average clustering strategy (UPGMA), Jaccard distance) was assessed. For the calculations the NuCoSa program (Tóthmérész, 1996) was employed.

Only the adults were considered in the material because of the paucity of larvae collected.

Results and Discussion

Results are presented in Table 1, 2, 3, 4. In Gödöllő1 site seven species were caught among which two were relatively frequent. Almost all of them (*Chrysoperla carnea*, *Chrysopa perla, Chrysopa formosa, Dichochrysa prasina, Chrysopa pallens)* belonged to the lacewings of a wide ecological

range and species favouring generally habitats with deciduous trees and shrubs (*Chrysopa viridiana*). The first two species (*Ch. carnea*, *Ch. perla*) predominated, amounting roughly 80% of the captured individuals. The seventh species (*Chrysopa phyllochroma*), species of low vegetation (especially in field crops) occurred only singly and very rarely. The species profile of Gödöllő2 was similar to the above. Seven species were captured consisting of species of wide ecological range (*Ch. perla, Ch. carnea*, *Ch. formosa, D. prasina*) and the woody vegetation preferring ones, *Dichochrysa flavifrons, Nineta flava*. At this place also *Ch. perla* and *Ch. carnea* prevailed, making out more than 75% of the individuals. Also *Ch. phyllochroma* was found singly. At Kartal1 site there were only two species, *Ch. carnea* and *Ch. formosa.* At Kartal2 occurred only *Ch. carnea* (Table 1).

The Gödöllő sites' individual density, species richness were much higher than those of Kartal. Their diversity and equitability values were also more important than the Kartal's estimates. The values of Gödöllő in case of abundance, species richness, diversity and evenness differed significantly from those of Kartal's. The Gödöllő sites' values do not differ significantly from each other and that is true also for the comparison of the Kartal sites (Table 2). Regarding the weekly catches, the diversity values are quite low but in case of the yearly estimates (calculated from summarized data) they are considerably higher. Considering the annual diversity the Gödöllő values differ significantly from those of Kartal, and the Katral values differ also from each other. Taking into account the scale dependent diversity characterization (Rényi diversity) the Gödöllő values are more diverse than those of Kartal in case of the frequent and the rare species. However, the Gödöllő assemblages cannot be separated, Gödöllő1 is more diverse in case of the rare, Gödöllő2 is more diverse in event of the frequent species (Figure 1). The similarity values processed by hierarchical cluster analysis showed a comparable picture like the diversity values: the assemblages of Gödöllő sites are alike to each other, and the relationship between those of the Kartal borders is analogous however, the Gödöllő hedges' assemblages at annual level differ remarkably from the lacewings of Kartal borders (Figure 2). The PCA gave a more detailed picture about the relationship of the lacewing assemblages. The similarity of the Kartal borders is indisputable but because of the divergent species composition, and dominance the Gödöllő lacewing groups differ considerably from each other (Figure 3). The dominance of *Ch. perla* is a new phenomenon because according to previous investigations made in Gödöllő and Budapest, this species'abundance was clearly more lower in semi-natural areas and gardens (Bozsik, 1994).

The influence of vegetation might have been substantial. Comparing the presence/absence values of the plant species found at the study places the cluster analysis verifies some inconsiderable similarity between the Gödöllő sites, and between the Kartal sites (Figure 4). Regarding the relationship of

the sites on the basis of the tree and bushy species another image appears which is more diverse, and which did not reflect the relationships of assemblages depicted above, where the biggest difference were found between the Gödöllő sites (Figure 5). There is one moment which may influence to a great extent the species richness and the abundance. This is the density of trees and bushes independently of the species composition. At the Gödöllő sites the tree density was considerable and similar which contributing to the various and favourable micro-climatic conditions could cause the greater species richness and abundance of the lacewing assemblages. In case of the Kartal sites where the woody vegetation was very scarce the variety of micro-climatic conditions could not be so wide, thus the values of structure parameters (species richness, abundance, diversity, evenness) were much smaller. It is difficult to compare these data with those of former reports because of the paucity of published information about lacewings in hedges. However, Duelli et al., (2002) showed that forest borders are real hotspots of lacewings, where their species richness and abundance can be considerable. They studied the relationship between the structure of forest edge and the catches of lacewing adults, from which they constructed the habitat preference categories of some Swiss lacewings. Unfortunately, only three categories could be applied for the Hungarian lacewings: ubiquist species (similar presence in most structures) for *Ch. carnea* s.l., contact zone species (presence in most peripheral structure) for *D. prasina*., canopy species (presence in topmost height) for *N. flava*. The other species have not been classified because their too rare or erratic distribution like that of *Ch. perla* or *Ch. pallens* (Table 4.).

Table 1. List of lacewing adults collected in the four sites in 1990-1992
([a] = percent of dominance values, - = species not found)

Species	Gödöllő1	Gödöllő2	Kartal1	Kartal2
Chrysoperla carnea	42.46[a]	35.07	75.00	100
Chrysopa perla	36.99	40.30	-	-
Chrysopa formosa	4.79	19.40	25.00	-
Dichochrysa prasina	2.74	0.75	-	-
Chrysopa pallens	6.16	-	-	-
Chrysopa viridana	5.48	-	-	-
Chrysopa phyllochroma	1.37	2.98	-	-
Nineta flava	-	0.75	-	-
Dichochrysa flavifrons	-	0.75	-	-
Total number of species	7	7	2	1
Total number of individuals	146	134	8	29

Table 2. Mean values of the structure parameters of lacewing assemblages in four hedgerows. (n = 16, samples of weekly catches. In brackets standard deviation. Means followed by the same letter within a column are not significantly different at P = 0.05 by two tailed t-test)

Sites	Number of individuals	Species richness	Diversity	Evenness
Gödöllő1	9.00[a] 6.088)	2.44[a] (±.413)	0.6016[a] (0.4889)	0.5969[a] (0.3901)
Gödöllő2	8.37[a] (6.956)	2.50[a] (1.265)	0.6363[a] (0.4823)	0.5710[a] (0.4064)
Kartal1	0.50[b] (1.155)	0.31[b] (0.704)	0.0785[b] (0.2157)	0.1132[b] (0.3113)
Kartal2	1.81[b] (5.089)	0.19[b] (0.403)	0.0000[b]	0.0000[b]

Table 3. Annual structure parameters of lacewing assemblages in four hedgerows. (Means followed by the same letter within a column are not significantly different at P = 0.05 by two tailed t-test)

Sites	Number of individuals	Species richness	Diversity	Evenness
Gödöllő1	146	7	1.3655[a]	0.7017
Gödöllő2	134	7	1.2664[a]	0.6508
Kartal1	8	2	0.5623[b]	0.8113
Kartal2	29	1	0.0000[c]	0.0000

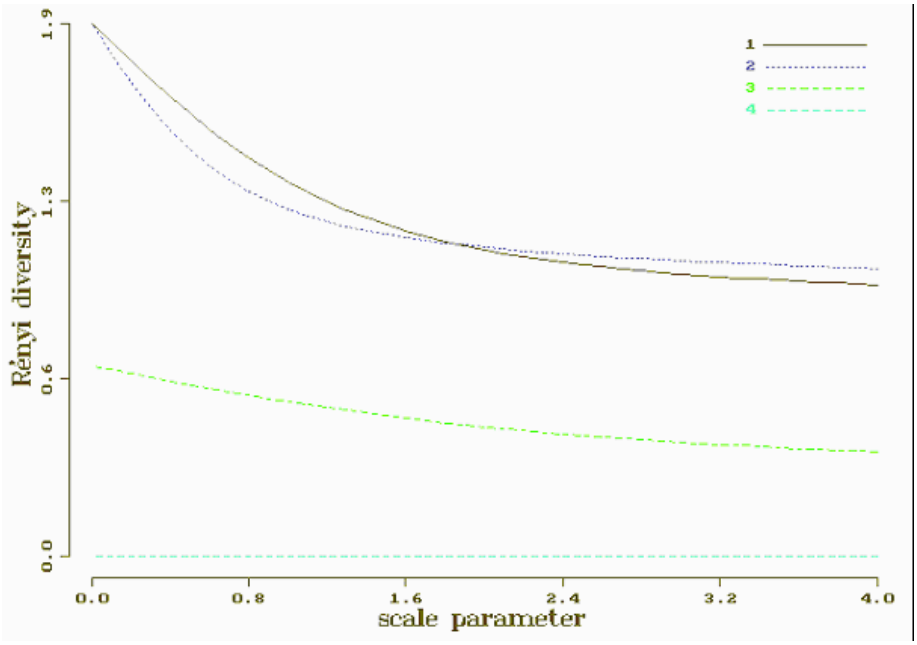

Figure 1. Rényi diversity of lacewing assemblages of study sites (small values of scale parameter correspond to rare species, big values to frequent species); 1: Gödöllő1; 2: Gödöllő1; 3: Kartal1; 4: Kartal2).

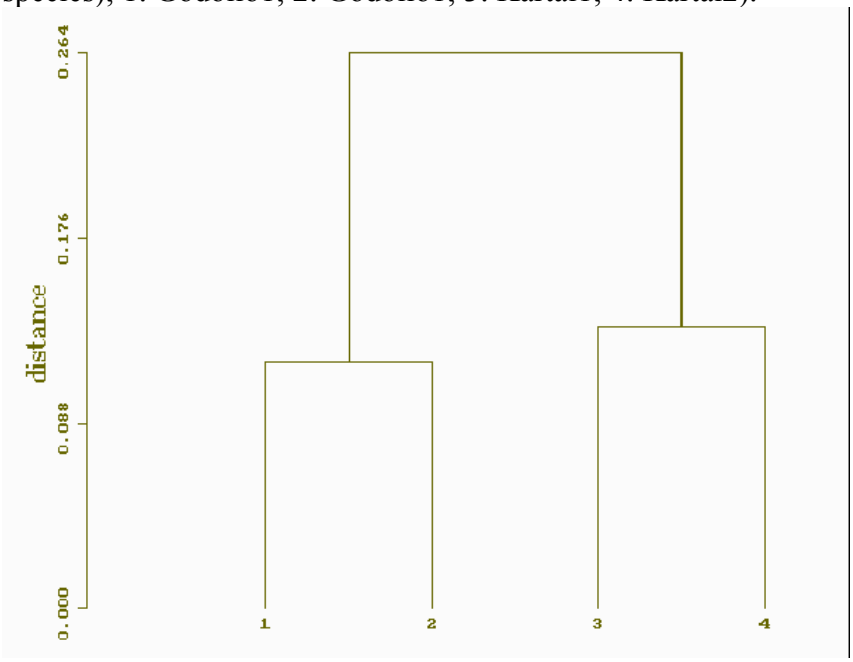

Figure 2. Similarity of lacewing assemblages of study sites (hierarchical cluster analysis; fusion: group average (UPGMA); quantitative distance: Matusita equation; 1: Gödöllő1; 2: Gödöllő1; 3: Kartal1; 4: Kartal2).

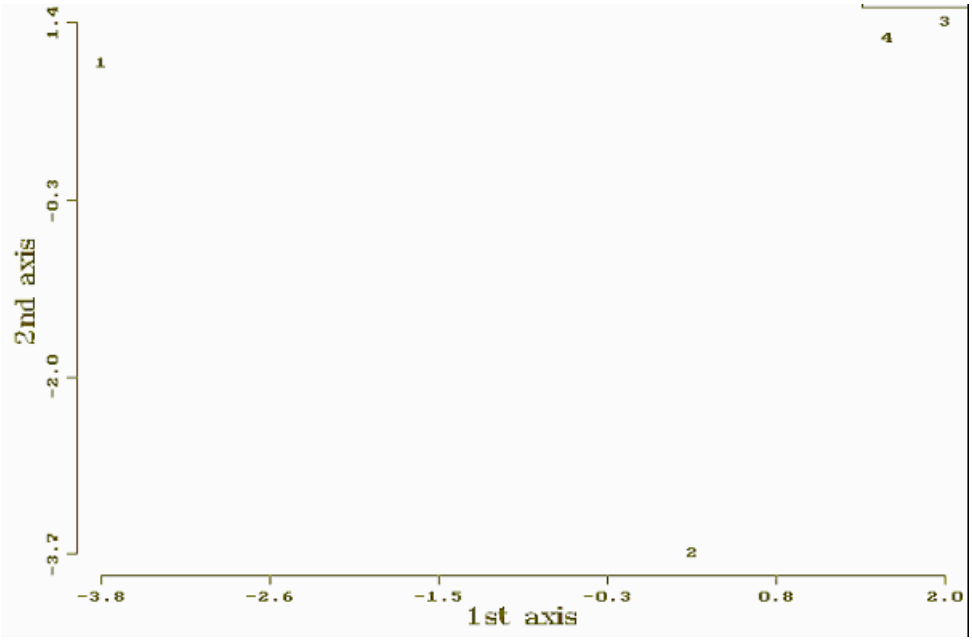

Figure 3. Principal component analysis of the lacewing assemblages of study sites (standardized, centered); 1: Gödöllő1; 2: Gödöllő1; 3: Kartal1; 4: Kartal2)

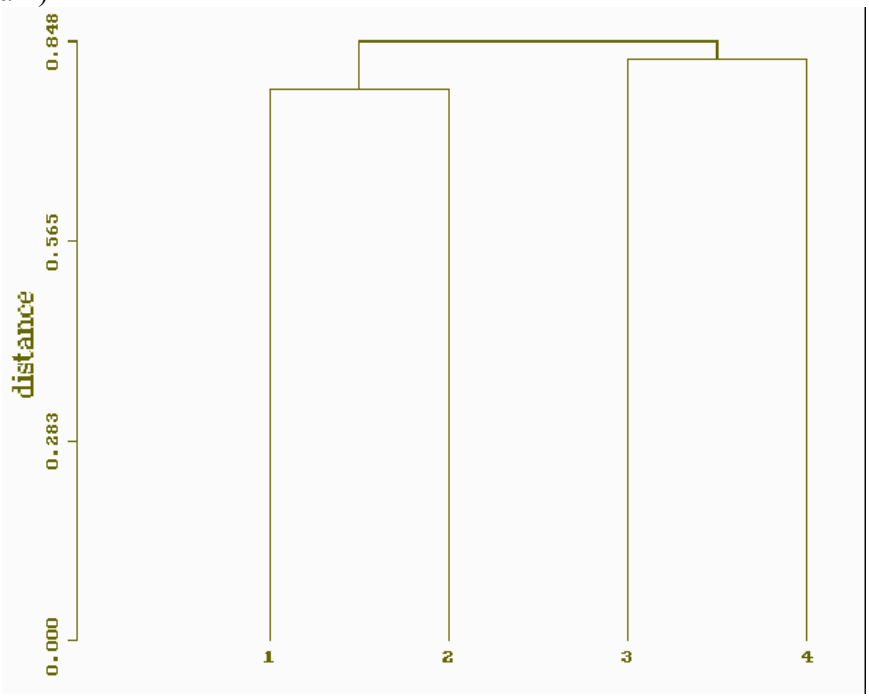

Figure 4. Similarity of the vegetation of study sites (hierarchical cluster analysis; fusion: group average (UPGMA); binary distance: Jaccard equation ; 1: Gödöllő1; 2: Gödöllő1; 3: Kartal1; 4: Kartal2)

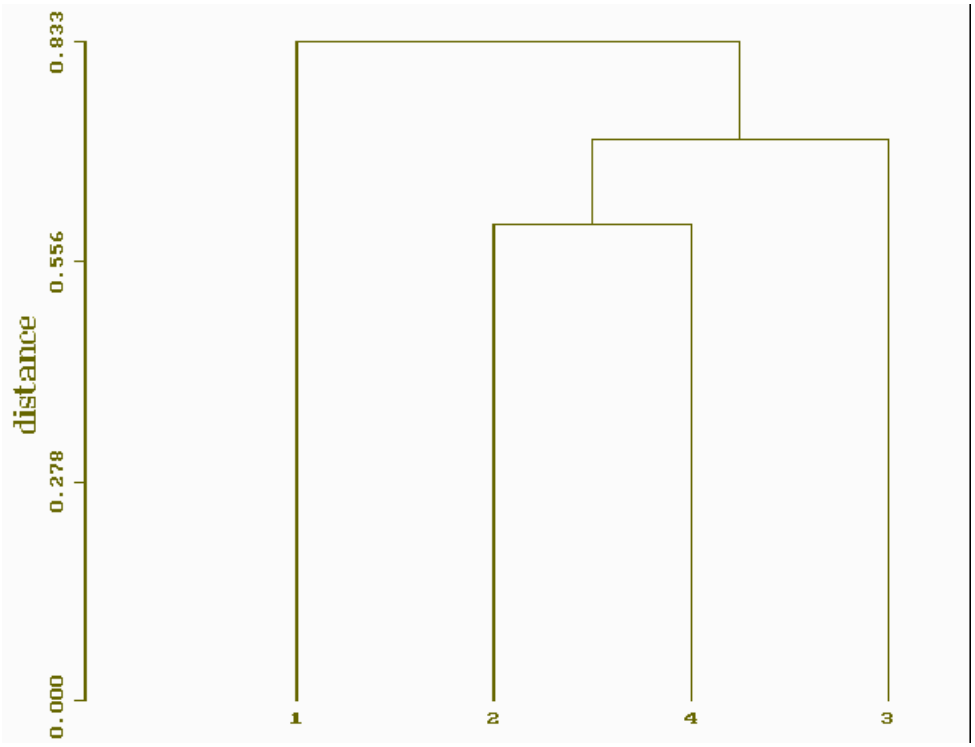

Figure 5. Similarity of tree and shrub stand of study sites (hierarchical cluster analysis; fusion: group average (UPGMA); binary distance: Jaccard equation; 1: Gödöllő1; 2 : Gödöllő1; 3: Kartal1; 4: Kartal2).

It has been shown that the presented hedges lacewing richness and abundance significantly surpassed those of the field borders with scarcely grown trees and shrubs. The very impact of hedgerows might be implemented rather by their structure (dense tree and shrub stand) than their tree and bush composition. This impact can be due to the hedge structure (forest edge mimicking structure with a herbaceous fringe) offering favourable micro-climatic conditions, sites for refugia, mating, oviposition, overwintering and wider choice of food as well as ecologic corridor for lacewings. As a consequence of these beneficial influence the maintaining and planting of agricultural hedgerows is a key question for the conservation and augmentation of lacewing biodiversity.

Table 4. Habitat preference of the lacewings collected. ([a]after Aspöck et al., (1980), [b]considering the forest edge structure /Duelli et al., 2002/)

Species	Types of habitat preference[a]	Types of habitat preference[b]
Chrysoperla carnea s.l.	all kinds of vegetation, eurytopic	ubiquist
Chrysopa perla	shrub belt, eurytopic	not classified
Chrysopa formosa	shrub belt	not classified
Dichochrysa prasina	tree and shrub, eurytopic	contact zone
Chrysopa pallens	tree canopy	not classified
Chrysopa viridana	tree and shrub (*Quercus* sp.)	not classified
Chrysopa phyllochroma	herbaceous vegetation	not classified
Nineta flava	tree and shrub (*Quercus* sp.), eurytopic	canopy
Dichochrysa flavifrons	tree and shrub, eurytopic	contact zone

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LACEWINGS' OCCURRENCE IN SOME HUNGARIAN HEDGEROWS AND FIELD EDGES

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Summary

The most important bordering semi-natural vegetational formations are in Europe the hedges or hedgerows. Their heterogeneous vegetation bordering cultivated areas supply sites for reproduction, overwintering and feeding for beneficial organisms, serving them as refugia, from which they can colonize the cultivated areas. Despite the importance of lacewings as natural enemies, only a few studies have been carried out concerning their presence in hedges and influence of vegetation on these insects. The study has shown that the presented hedges lacewing richness and abundance significantly surpassed those of the field borders with scarcely grown trees and shrubs. The very impact of hedgerows might be implemented rather by their structure (dense tree and shrub stand) than their tree and bush composition. This impact can be due to the hedge structure (forest edge mimicking structure with a herbaceous fringe) offering favourable micro-climatic conditions, sites for refugia, mating, oviposition, overwintering and wider choice of food as well as ecologic corridor for lacewings. As a consequence of these beneficial influence the maintaining and planting of agricultural hedgerows is a key question for the conservation and augmentation of lacewing biodiversity.

WEED SCIENCES SESSION

DETECTION OF COMMON RAGWEED (*AMBROSIA ARTEMISIIFOLIA* L.) REFLECTANCE SPECTRUM BY MEANS OF FIELD MEASUREMENTS

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The paper describes measurements carried out by ASD Filed Spec in two different Hungarian regions (Jánossomorja, Tiszakécske). We tried to specify the reference spectra of common ragweed (Béres 2003) and background vegetation and results of analysis. The main scope of the experimental measurements was the detection and mapping of the reflectance spectrum of common ragweed in order to elaborate the airborne-remote sensing methodology for weed monitoring. First element of the methodology was to specify the reference spectra of the target object. The reflectance measurements were taken in the wavelength range of 0.35–2.5 microns comprising the visible- and near-infrared wave bands (VIS-NIR). The elaboration of the methodology involves a feasibility study of hyperspectral imaging and the adaptation of hyperspectral methods for using them in the classification of multispectral images taken by satellites (Itzerott–Kadenn 2006, Lichti et al. 1997).

The measurements were carried out in cooperation with the University of Natural Resources and Applied Life Sciences (Institute for Surveying, Remote Sensing and Land Information) also in 2006. Main aims of the measurements were to define typical reflectance spectrum for common ragweed (Kardeván et al. 2004) and sunflower in order to study spatial heterogenity of by ragweed "polluted" agricultural areas in two different phenological states: at the beginning and end of July. The processed measurement results were used to "teaching" classification algorithms for images (to find end-member spectra) sensed by satellites (LANDSAT5, SPOT5, ASTER). To fulfil our aims we measured in pure by common ragweed covered- and by sunflower covered spots and by random sampled areas (totally 16 spots). We analysed the weed-sunflower-soil components in percent distribution. The relative distribution of weed-soil-culture plant were evaluated with the method of Balázs-Újvárosi (Tóth–Spilák 1998, Reisinger 2001, Reisinger et al. 2001). Last three years we have build a

common ragweed spectral library for different phenological states that may be a very good scientific background for others to analyse ragweed.
Our future plan is to be able to detect invasive weeds by satellites or airborne hyperspectral remote sensors in high spatial resolution (Vrindts 2000). Of course by this way the first investigations are and will be focused on common rag weed. During digital image processing the following methods were applied: supervised classification, clustering, target detection, image transformation (Cruse et al. 1993, Tamás 2004, Burai–Pechmann 2005, Jung et al. 2005, Kis Papp–Jung 2005).

Materials and Methods

- In the year of 2003: Application of supervised classification (with teaching points) without atmospheric correction. Connecting DGPS measurements with by sensor calibrated satellites images.
- In the year of 2004: Studying of reflectance spectra for agricultural plants and typical weeds in order to distinguish them.
- In the year of 2005. Defining representative spectra by field spectroradiometers for agricultural areas in different classification categories for different phenological periods of common ragweed.
- In the year of 2006. New technological and methodological experiment to understand the atmospheric effect on the field measurements.

The used spectroradiomer was an ASD Field Spec Pro FR in the wavelength range of 0.4-2.4 μm. The IFOV of the spectroradiometer was 28˚. In the IFOV the projected or sensed area was 2-5 m in diameter depended on height.
To calculate the reflectance factor for the target object we used a white spectralon. For every measurement we defiend the plant-soil cover in relative distribution by the Balázs-Újvárosi Method. During sampling we made photos according to IFOV of spectrometer for every spot for after-processing feedback. This feedback was of great importance at desktop analysis to decide where shadow or other disturbing effect was.
There was a new methological approach in our investigation. We combined the so called “subjective” Balázs-Újvárosi Method with a spectrometric measurement process. This combination delivered good result at final comparisons. The simple target detection was complemented with a sundisc measurement that was very promising in atmospheric correction but it is behind the scope of the present paper.

Spectral measurements in 2005, 2006 were supported by FÖMI (Institute of Geodesy, Cartography and Remote Sensing). The project was sponsored in the framework of NKFP named ‘Elaborating of Pollen Information System’.

Results

In Figure 1 can be seen that common ragweed has a typical reflectance spectra and spectral anomalies are strongly influenced by the existence of other plants in sensed areas. This phenomenon is well known and can be recognised in our experiments as well. At the right site of Figure1 there is a list of different relative plant distribution (evaluated with the Balázs-Újvárosi Method) where ‘Pf’ means ragweed and other symbols refer to other weeds that have effect on ragweed spectrum also.

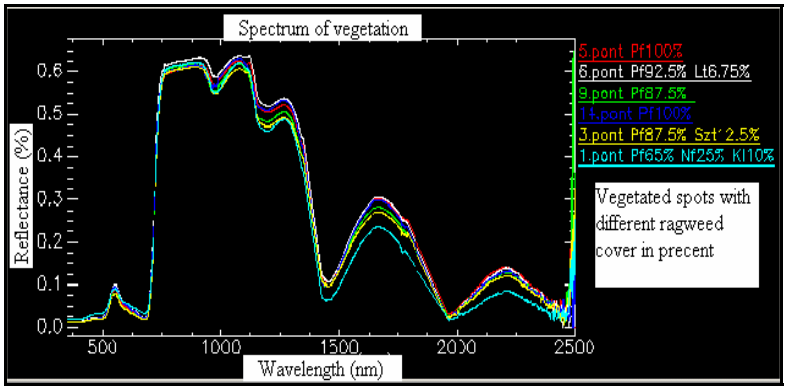

Figure 1. Vegetation spots in different relative distribution with dominant ragweed population (Pf refers to *Ambrosia*)

Figure 2 shows the real differences between common ragweed and sunflower in the case when sensed area was homogeneous. So we can see that there is a very good chance to find a gap in the spectra to distinguish ragweed from sunflower by means of remote sensing.

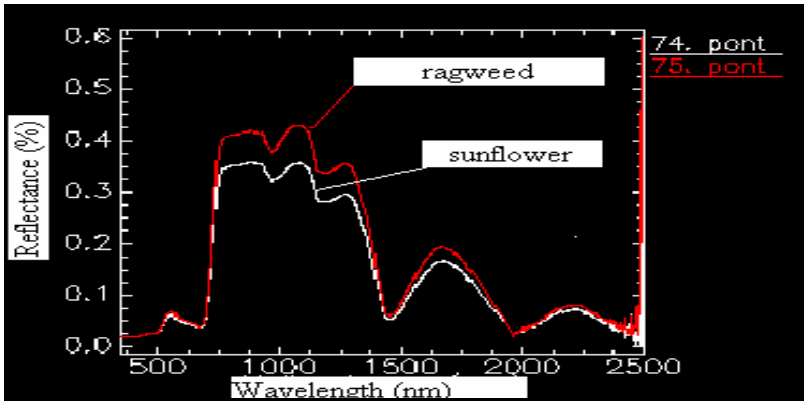

Figure 2. Reflectance spectra of homogeneous sunflower and ragweed canopy

It is good sign to be able to automate a pattern recognition process because this difference is well recognisable. It is widely known that homogeneous population are very seldom in the nature and heterogenity is more typical but in a theoretical approach it is recommended to clarify the differences.

In Figure3 we collected reflectance spectra for four situations. It is very interesting to state that a sunflower population with and without weeds how rapidly decreases in reflectance, especially in the spectral range 600-1400 nm. It is the visible and near-infrared segment of the spectrum (Gitelson et al. 1996). For our investigations it was of increasing importance to map the spectral properties of different vegetation.

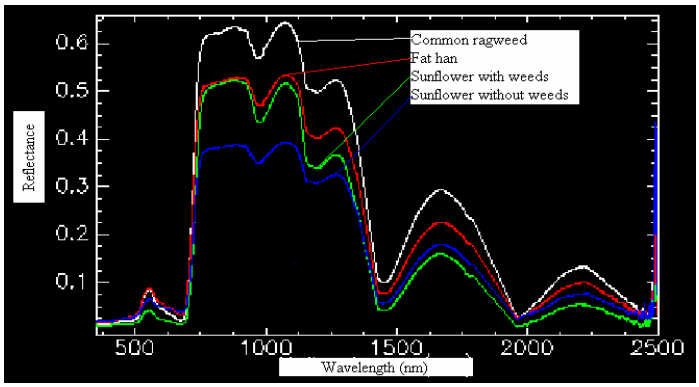

Figure 3. Differences in homogeneous and heterogenic reflectance spectra

In Figure 4 was presented the most important result for the practice or precision farming because in detecting common ragweed we were seeking for unique signs in the spectra. Spatial and temporal properties were the keys for recognising ragweed in mixed vegetated area. After our results we

concluded that the phenological state was a very promising factor in detection and recognition of common rag weed in a sunflower population. The spectral differences were much more expressed on 26.06., than on 06.06.

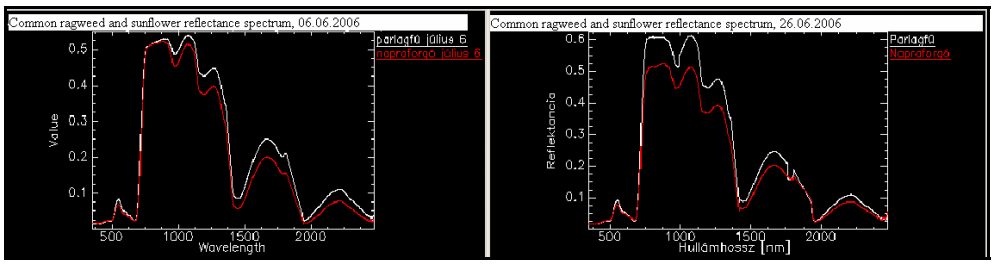

Figure 4. Differences in reflectance spectra caused by phenology (06.06 and 06.26.2006)

According to our future plans we would like to detect common rag weed by satellites. In our investigation we used ENVI 4.0 software that had a build-in algorithm that could recalculate the original hyperspectral database and resampled it to a given satellite band distribution. We resampled the measurements for three satellites: ASTER, SPOT5, LANDSAT5.
After resampling can be stated, that phenological differences in reflectance spectra can be well distinguished by space-borne sensors also. The graphical result are shown in the Figure5.

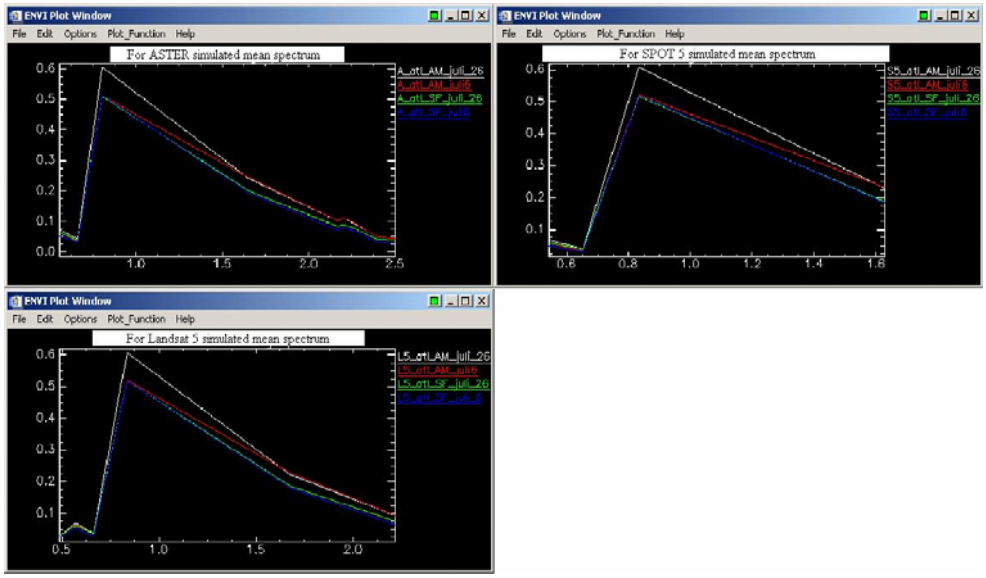

Figure 5. Spectral resampled spectra for ASTER, SPOT5 and LANDSAT5 satellites.

In our investigation we found unique spectral signs for detecting common rag weed in a sunflower population. Our measurements were carried out by field spectroradiometer that had very high spectral resolution. Our best results were found to be on 26.06.2006 so the right phenological point has priority in the successful detecting process. This technology can be commonly known, used and relatively cost effective when measurements will be taken by satellites. For satellites resampled results we concluded also that phenology is very relevant but applicable in the decision process.
We would like to continue the field measurements and to involve more satellite images. As present problem muss be mentioned that atmospheric effect is very disturbing in the field also and real atmospheric correction solution are in process nowadays.

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DETECTION OF COMMON RAGWEED (*AMBROSIA ARTEMISIIFOLIA* L.) REFLECTANCE SPECTRUM BY MEANS OF FIELD MEASUREMENTS

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Summary

We specified the reference spectra of common ragweed and background vegetation and presented the results of analysis.
In our investigation we found unique spectral signs for detecting common rag weed in a mixed sunflower population. Our measurements were carried out by field spectroradiometer that had very high spectral resolution. Our best results were found to be on 26.06.2006 so the right chosen phenological state has priority in the detecting process. The technology will be commonly known, used and relatively cost effective when measurements will be taken by satellites. For satellite images resampled results we concluded that phenology is very relevant in the decision process.

We would like to continue the field measurements and to involve more satellite images. As present problem muss be mentioned that atmospheric effect can be very disturbing in the field also and real atmospheric correction solution are in process nowadays.
We hope that our investigations offer new perspectives in weed monitoring and management.

RESULTS OF WEED SURVEY IN WHEAT CROP MANURING FIELD EXPERIMENT

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The most important component of the plant production technology is the proper subsequent delivery of nutrients. The winter wheat is one of the cereals, which have the highest requirements and which most reacts to the nutrient supply (Szentpétery et al., 2005). The nutrient demands of weeds, and their capacity for self-accommodation to the differing nutrient levels is considerably different.

The weeds, just like the cultivated plants absorb the nutrients and water from the soil to build up their organism. A competition will develop for the basic growing and developing conditions. The weeds take up big amount of water during their growing. Also their transpiration rate is very high (Kádár, 1983). A competition takes place when two or more organisms seek after a given factor to satisfy their special demands in a situation, when the supply of this factor is lower than the summarized demand of the organisms (Milne, 1961). That is why the cultivated plants are able to tolerate the weeds for a certain time. If we could keep a crop weed free for an adequate time, the crop then would be able to shadow and suppress the emerging weeds (Rademacher, 1966). The competitive ability of the cultivated plants can be increased by better agrotechnique and by plant breeding (Berzsenyi, 2000).

The soil fertility of a field will be determined mainly by its organic matter content. The organic matter mineralization, and by this the mobilization of nitrogen can be influenced by a proper soil use, plant rotation and soil management. After all, the continuous sustaining of soil fertility, good soil condition can be promoted by a proper way used agrotechnique (Kismányoky, 1994).

The proper nutrient delivery, or rather fertilization may promote the development, competition ability and weed suppressing ability of the cultivated plant. The biodiversity of weeds makes possible for them using both the nutrient-poor and nutrient-rich soils. An unwork-menlike fertilization may be a weed propagating factor if it results in decreasing of shadowing ability of the cultivated plant, since from the wide weed spectrum those weeds start to develop and grow, which are most able to use the extreme nutrition situation. The skilled fertilizing results in a better covering of the soil by cultivated plant and a minimalized weed presence (Kádár et al., 1999). The aim our trial was to investigate the weed presence

in a long term fertilizing trial in wheat, in relation to the way of nutrient supply (NPK, NPK+FYM, NPK+haulm rest) and to the nitrogen level.

Materials and Methods

The weed survey were made on a long term fertilizer trial of the Experimental Station of University of Pannonia, Department of Plant Protection and Soil Science. This long term bifactorial trial with split-plot arrangement in three repetition were started in 1983. The plot size was 6×8 m = 48 m^2. The test plants were maize, winter wheat and barley.
Treatment A: nutrient: NPK, NPK+35t/ha FYM, NPK+haulm rest.
Treatment B: N kg/ha^{-1} N_0 - N_4 (0, 50, 100, 150, 200), and 100 kg P_2O_5 $ha^{-1}$and 100 kg K_2O. The time of wheat sowing was October 10, 2005. No weed killing were made up to the time of our investigations, which made possible the examination of early weed density in relation to the nutrient delivery (NPK, NPK+FYM, NPK+haulm rest), and level of N-delivery. The weed survey were made using the Balázs-Ujvárosi method (Reisinger, 2001).

Results

We have found altogether 15 weed species in the trial. In the NPK treatments were 13 species (Table 1), in the treatment NPK+organic manure 11 species (Table 2), and in the NPK+haulm rest treatment 9 species (Table 3) found.

Table 1. The dominance order of weed species on the plots of NPK treatment

	Weed species	Covering value*	Frequency of occurrence
1	*Veronica hederifolia* L.	0.81	14
2	*Abutilon theophrasti* MEDIC.	0.20	14
3	*Chenopodium album* L.	0.18	10
4	*Consolida regalis* S. F. GRAY	0.11	9
5	*Ambrosia artemisiifolia* L.	0.03	5
6	*Sonchus asper* (L.) HILL.	0.03	4
7	*Bilderdykia convolvulus* (L.) A. LÖVE	0.02	3
8	*Stellaria media* (L.) VILL.	0.02	3
9	*Chenopodium hybridum* L.	0.01	2
10	*Taraxacum officinale* WEB.	0.01	2
11	*Raphanus sativus var. Oleiformis*	0.01	2
12	*Polygonum persicaria* L.	0.01	1
13	*Cirsium arvense* (L.) SCOP.	0.01	1
Total weed covering:		**1.45**	**-**

*in the average of the N-treatments

Table 2. The dominance order of weed species on the plots of NPK+FYM treatment

	Weed species	Covering value*	Frequency of occurrence
1	*Abutilon theophrasti* MEDIC.	1.45	15
2	*Veronica hederifolia* L.	1.35	14
3	*Chenopodium album* L.	0.92	13
4	*Consolida regalis* S. F. GRAY	0.06	9
5	*Ambrosia artemisiifolia* L.	0.05	5
6	*Stellaria media* (L.) VILL.	0.02	3
7	*Polygonum aviculare* L.	0.02	3
8	*Taraxacum officinale* WEB.	0.02	3
9	*Chenopodium hybridum* L.	0.01	1
10	*Oxalis europae* JORD.	0.01	1
11	*Raphanus sativus var. Oleiformis*	0.01	1
Total weed covering:		**3.91**	-

*in the average of the N-treatments

Table 3. The dominance order of weed species on the plots of NPK+ haulm rest treatment

	Weed species	Covering value*	Frequency of occurrence
1	*Veronica hederifolia* L.	3.46	15
2	*Chenopodium album* L.	2.73	15
3	*Abutilon theophrasti* MEDIC.	0.58	13
4	*Ambrosia artemisiifolia* L.	0.20	5
5	*Raphanus sativus var. Oleiformis*	0.12	4
6	*Taraxacum officinale* WEB.	0.05	3
7	*Bilderdykia convolvulus* (L.) A. LÖVE	0.05	2
8	*Chenopodium hybridum* L.	0.03	2
9	*Consolida regalis* S. F. GRAY	0.03	2
Total weed covering:		**7.24**	

*in the average of the N-treatments

Most of the weeds (8 species) were belonging to the T_4 life cycle type group. The first in the order of dominance was *V. hederifolia* on the treatments fertilizers+haulm rests, while on the treatment fertilizer+FYM the *A. theophrasti* was dominant. Considerable differences between the treatments existed in the weed density and weed dominance sequence. It has to be noted, that in all the three fertilizing treatments the same three weed

species occupied the first places, but their order were not the same. In the treatment fertilizer only and fertilizer+haulm rest *V. hederifolia* was the first, while in treatment fertilizer+FYM the *A. theophrasti*.
In the NPK treatment the *V. hederifolia* was the dominant weed species with an average covering value of 0.81%, the second was the *A. theophrasti*, and the third was *C. album*. In the treatment with FYM the A. theophrasti showed the highest covering value, 1.45%. The second was again the *V. hederifolia* and the third the *C. album* with 0.92% covering. On the plots of treatment NPK+haulm rest, similar to that of the treatment NPK only, the *V. hederifolia* was the most dominant, here with a value of 3.46%, the second was *C. album* (3.46%), and third the *A. theophrasti* (0.58%).
We observed statistically verified differences in the weed density in relation to the different ways of fertilizing (Table 4). The highest average weed covering value (7.24%) was observed on plots of the treatment NPK+haulm rest. The second highest average value (3.91%) was on plots of treatment NPK+FYM, while the smallest (1.45%) in the case of treatment with only NPK.

Table 4. Average weed coverings of the treatments

	Weed covering values		
N-	**Fertilizer treatments**		
treatments	**NPK**	**NPK+FYM**	**NPK+haulm rest**
N_0	1.6	1.96	3.26
N_1	1.12	4.7	4.37
N_2	1.64	3.6	9.1
N_3	1.16	4.87	9.77
N_4	1.74	4.43	9.68
average	**1.45**	**3.91**	**7.24**
$LSD_{5\%}$	**1.14**		

The differences among the treatments were significant. The average weed covering value of the treatment NPK+FYM was 2.7 times higher, than that of treatment NPK only, and the value of NPK+haulm rest was nearly 5-times higher. The difference between NPK+FYM and NPK+haulm rest was nearly doubled.
Within the different fertilizing treatments only the treatment NPK+haulm rest was, where the different N-doses caused significant differences in the weed covering rate. In relation to the N_0 and N_1 doses, the doses N_2, N_3 and N_4 caused significant, twofold, threefold higher weed covering rates. Between the N_0 and N_1 treatment there was no significant difference in weed covering, and the differences among N_2, N_3, N_4 treatments were also small. We can state, that in these last treatments the weed covering had an

increasing tendency, as a result of increasing amount of N-doses, and this tendency was in some case statistically verified.

Conclusions

Based on the results it can be stated, that the extent of weed covering increased on those plots, where also organic matter (farmyard manure or haulm rest) were plugged into the soil. In the time of the end of wheat tillering the difference is considerably bigger – 2.7 to 5-fold – in relation to the values of plots getting mineral fertilizers only. Within the main treatments the increasing doses of N resulted in significant weed covering increase in that treatment, where the haulm rest were plugged into the soil. The plots getting higher amounts of N-fertilizer, showed higher weed covering.

Acknowledgements
Thanks are due to professor Dr. Tamás Kismányoky for giving the possibility to join to his long-term field trial. This research work was supported by the program of the National Fund for Scientific Research, No. OTKA K60314 and T 46845.

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RESULTS OF WEED SURVEY IN WHEAT CROP MANURING FIELD EXPERIMENT

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Summary

The weed survey were made on a long term fertilizer trial of the Experimental Station of University of Pannonia, Department of Plant Protection and Soil Science. This long term bifactorial trial with split-plot arrangement in three repetition were started in 1983. The plot size was 6×8 m = 48 m^2. The test plants were maize, winter wheat and barley.
Treatment A: nutrient: NPK, NPK+35t/ha FYM, NPK+haulm rest.
Treatment B: N kg/ha^{-1} N_0 - N_4 (0, 50, 100, 150, 200), and 100 kg P_2O_5 $ha^{-1}$and 100 kg K_2O

The time of wheat sowing was October 10, 2005. No weed killing were made up to the time of our investigations, which made possible the examination of early weed density in relation to the nutrient delivery (NPK, NPK+FYM, NPK+haulm rest), and level of N-delivery. The weed survey were made using the Balázs – Ujvárosi method at the end of wheat tillering.

Based on the results it can be stated, that the extent of weed covering increased on those plots, where also organic matter (farmyard manure or haulm rest) were plugged into the soil. In the time of the end of wheat tillering the difference is considerably bigger – 2.7 to 5-fold – in relation to the values of plots getting mineral fertilizers only. Within the main treatments the increasing doses of N resulted in significant weed covering increase in that treatment, where the haulm rest were plugged into the soil. The plots getting higher amounts of N-fertilizer, showed higher weed covering.

POTENTIALS OF CHEMICAL CLEARING OF "ENERGY WILLOW" (*SALIX VIMINALIS* L.)

Attila Kondor[1] – István Lenti[2]

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Because of the decrease of the fossilic energy resources and the threats and unresolved problems of atom energy, efficiency of renewable energy resources are becoming more and more prominent. Despite of the fact that renewed energy resources consumption has a relatively long history, its contribution to the global energy utilization is still fairly moderate.
According to conditions in Hungary in 2003, renewed energy consumption represents a 3,6% partial produce within the total energy use (Tar et al., 2003). Under Kyoto Protocol and European Union accession Hungary agreed on doubling this value by the year of 2010.
It is essential to recognize that these co-national commitments are accord with our own national aspirations, particularly in decreasing the level of environmental pollution resulting from energy processing by exploiting renewable energy resources. Thus, we could also temper the economical dependency on energy resource import of our country.
One of the greatest potentials of renewable energy resources in Hungary is that of biomass (Gonczlik et al., 2005), since our land show off very distinguished natural endowment for the energetic employment of this resource material.
A plant species bearing outstanding energy-providing qualities amongst biomass energy resources is "energy willow" (*Salix viminalis* L.).
A hybrid plant has been improved in Japan which answers a certain floral attribute characterizing plants with alternative energy resource potentials; it grows most vigorously. Hence the name "stick willow" used among cultivators. As far as we know this plant grows 3-5 cm daily, and its specific yield is 20-40 TN/ha/Y. Its high salicylic alcohol content provides excellent heat value, and the combustion heat of its twig is 29,2 MJ/kg (Kiss, 2005).
willow plantations are often elements of softwood parks and juvenile coppices in flooded and shelfy river-flat areas. Plant associations in these areas show off a relatively plain and bare specific assortment. They frequently constitute complexes with other plant species on riverbank zones. Condition of survival for willow are assured by overflowing, however it develops fairly well on extreme vegetative sites as well – e.g. in case immersed in water in springtime or growing on arid lands with short water

supply (Borhidi A. 2003). Soó (1951) classified poplar (*Populus*) and cottonwoods (*Salix*) genii into willows family *Salicaceae* reciting individual species within it referring to their variants and hybrids. In his taxonomic work Borhidi (1995) placed poplars genus within *Salicales* order and willows family *Salicaceae*. Simon (2000) treated willows family *Salicaceae* and *Salix* genus similarly in which he enumerated 13 species.

Materials and Methods

Our research of cultivation technology or more specifically plant reservation was accomplished in „Szalka-Pig Ltd." in Mátészalka. After necessary professional consideration its proprietor in 2005 decided to procure *Salix viminalis* L. willow species by import for a plantation on his humid and waterish plough lands for energy accumulation purposes. He enlarged this area with another 43 ha in 2006. The owner then decided to plant the species in not only adherent (K_A 70), but also more loose structured and sandy soils (K_A 30-35).
The applied planting system was the following: 75 x 45-50 cm stem and bed space in twin rows, followed by a 110 cm width bed space, then again 75 x 45-50 cm planting space in twin rows followed by a 260 cm width cultivation path. Thus 18-20 thousand plants were settled on 1 ha (see Figure 1, 2.)

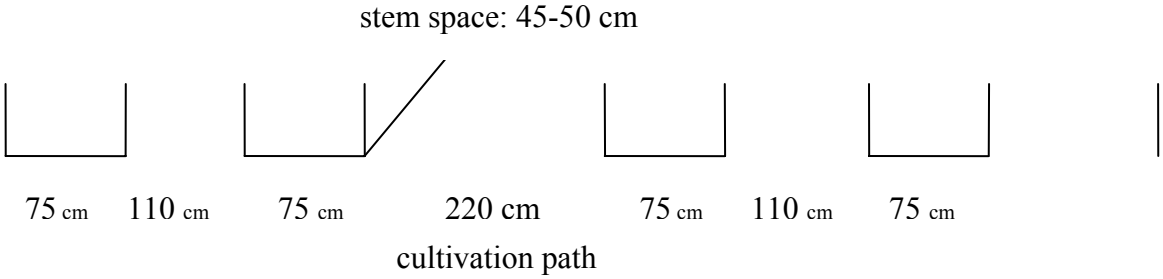

Figure 1. Applied planting system in a *Salix viminalis* L. plantation

Figure 2. Bunched willow-cuttings ready for implantation

While inventing growing technology for this plant species, we faced up challenges in two problematic matters; plant reservation and gathering. This very essay intents on outlining plant reservation problems of clearing.
The most problematic question is clearing, which is consequent to the act of planting. We took up as responsibility to elaborate a pre-emergent clearing method for this willow species, as a clearing treatment completed during this phase would solve duties alike for a whole planting year.
For pre-emergent clearing experiments we marked out 500 m^2 areas of either loose and sandy, or adherent alluvial soils.
Applying the under-mentioned herbicidal agents, we calibrated the post-plantation field experiments for a pre-emergent clearing:

In 2005;

1. terbutilazine	+S-metolachlor,	Click FL	+	Dual Gold 960 EC
2. mezotrione	+S-metolachlor,	Calisto 4 SC	+	Dual Gold 960 EC
3. pendimetaline	+S-metolachlor,	Stomp 330	+	Dual Gold 960 EC
4. oxyfluorfen	+S-metolachlor.	Goal 2 E	+	Dual Gold 960 EC

In 2006;

1. terbutilazine	+S-metolachlor,	Click FL	+	Dual Gold 960 EC
2. mezotrione	+S-metolachlor,	Calisto 4 SC	+	Dual Gold 960 EC
3. pendimetaline	+S-metolachlor,	Stomp 330	+	Dual Gold 960 EC

On selecting appropriate agent combinations we inquired notable specialists, whose observations and suggestions were essential in the matter.
We selected the adopted herbicides according to the permission certificate; in a low value on sandy areas and in a high value in case of adherent soils. We find it necessary to mention that there is no available licensed herbicide for clearing willows.
We assessed treatments in 3 occasions fortnightly. The first assessment took place on 30th July. We took weeds samples and compared the data with one another and also with those mechanically treated plantations.

Results

It was established that clearing treatments accomplished pre-emergently did not spoil cultivated crops in any combination or dosage. The effect of herbicides showed different results.
Dominant weed variants planted on loose, sandy soils as a treatment thinned out but did not decay. Applying high rate dosages on more adherent soil ended up in better results.
Several effects of different chemicals are contained in Figure 1. On the basis of all three treatments we can state that bearbind (*Convolvulus arvensis*) does appear, moreover that, compared to the control, pre-emergent clearing generated good results (see Figure 3).

Table 1. Clearing effect of different herbicidal combinations on „energy willow" plantations

chemical combinations	observation 1.	observation 2.
Click FL + Dual Gold 960 EC	**Appeared**: goosefoot family, ragweed. **Did not appear**: monocotyledonous weeds.	**Appeared:** monocotyledonous weeds in traces, ragweed sparsely, goosefoot family on an average coverage.
Calisto 4 SC + Dual Gold 960 EC	**Appeared**: Of monocotyledonous weeds: Setaria glauca. **Did not appear:** dicotyledonous weeds.	**Appeared**: hungry rice in a great amount. Of monocotyledonous weeds: goosefoot family. **Did not appear:** ragweed.
Stomp 330 + Dual Gold 960 EC	**Appeared:** ragweed. **Did not appear:** monocotyledonous weeds.	**Appeared:** ragweed on a considerable scale. **Did not appear:** monocotyledonous weeds were still not trackable.

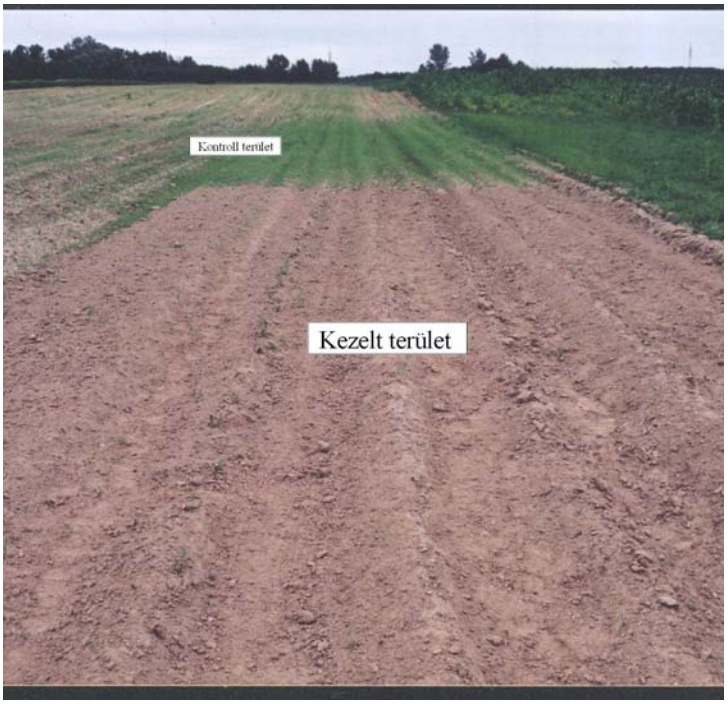

Figure 3. The control (kontroll) and the treated (kezelt) area

After the first treatment monocotyledonous weeds appeared in traces on the area, while ragweed (*Ambrosia artemisiifolia*) appeared scarcely beside the coverage of the members of the Chenopodiaceae family (*Chenopodium* spp.) hungry rice (*Digitaria sanguinalis) s*cattered on a large scale on the plantation after the second treatment. Of the diphyllous plants goosefoot family appeared. Ragweed could not be tracked down. The treatment repeated for the third time ragweed appeared in a considerable amount. Monocotyledonous weed could not be tracked down.

Summary

Agent combinations used in pre-emergent clearing field experiments with "energy willow" (*Salix viminalis* L.):

1. terbutilazine + S-metolachlor,
2. mezotrione + S-metolachlor,
3. pendimetaline + S-metolachlor,
4. oxyfluorfen + S-metolachlor.

These complete clearing experiments did not harm the cultivation plants in any combination or dosage. Treated on loose and sandy soil, dominant weed

species, though thinned out, did not decay. The applied higher dosage on more adherent soil resulted in fine outcome. Herbicides employed in this experiment were suitable for a chemical clearing of the willow. This experiment must be reinforced by long-term experiments.

Relatively resistant weed species in willow plantations:

- bindweed (*Convolvulus arvensis*),
- ragweed (*Ambrosia artemisiifolia*),
- marijuana or hemp (*Cannabis sativa*),
- goosefoot species (*Chenopodium spp*.).

Based on these results, recorded field experiments could establish the elaboration of chemical clearing methodology of the willow.

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POTENTIALS OF CHEMICAL CLEARING OF "ENERGY WILLOW" (*SALIX VIMINALIS* L.)

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Summary

A paper about weed control possibilities of energy willow (*Salix Viminalis* L.)

NEW POST EMERGENT HERBICIDE APPLICATION POSSIBILITY IN MAIZE USING THE PROTOX-INHIBITOR HERBICIDE, FLUMIOXAZINE (PLEDGE®)

András Horn – Ferenc Jáger

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It is well known that the herbicide active ingredient flumioxazine (Pledge, Sumi-Soya) belonging to the mode of action: protox-inhibitors, is widely used in several crops, among other in maize (corn) as preemergent herbicide.
Flumioxazine is effective mainly against dycotyledonous weeds. Based on this, under Hungarian conditions it is applied in tankmixture with graminicide herbicides, most of them belonging to the cloracetanilid group (acetochlor, dimetenanid, pethoxamid).

Considering several factors, Summit-Agro started trials to check the possibility to use Flumioxazine not only as preemergent but also as postemergent herbicide in maize(corn).
---- Under praxis condition several time the preemergent application is delayed. It was recommended to check the risk of postemergent application.
---- Postemergent application is used on more than 50 % of the total territory.
---- The cost/hectare of the presently used postemergent herbicides is rather high.

Based on several information trials, and registration trials in Hungary the results can be summarized as follows:

A. Flumioxazin (Pledge 50 WP) can be used as postemergent herbicide in maise(corn) with the dosage: 80 g/ha at the fenological stage of 3-5 leaves of maize(corn).

B. Slight phytotoxicity on maize can be observed always after the treatment, but this damage never influenced the yield.
Full recovery of maize(corn) can be expected within 1- 2 weeks.

C. Merit of the treatment:
------Reasonable cost/ha.
------Flumioxazine (Pledge) is killing (as contact herbicide) all , already germinated weeds.

------Flumioxazine (Pledge) is forming a herbicide –layer (film) on the soil surface, and it is killing also the weeds germinating after the treatment.

Due to the fact that :
---in Hungary the sown area of maize and sunflower is extremely large.
---Furthermore, Flumioxazine is used preemergent and postemergent in sunflower.
Flumioxazine is selective on sunflower under Hungarian conditions.
Based on above facts in case of sowing maize after sunflower, sunflower can be one of most dangerous weed in maize.
To solve this problem Summit Agro developed postemergent (also Flumioxazine containing) tank mixture in maize, to kill weed sunflowers as well.

COMPETITION OF SUNFLOWER AND MAIZE WITH SEVERAL WEED SPECIES

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Weeds endanger safety of crop production reducing seed yield volume and quality in every year. Their effects depend on crops, weeds associations, several environmental factors and cropping systems.

Dominance conditions of weeds are depending on cultivated crops and other local factor, furthermore changing during years or tens of years. These changings can be followed, so future processes could be forecasted. There were significant changing in dominance conditions both in arable lands and plantations (Szőke 2001, Solymosi 2005, Dancza et al. 2006): Occurrence of some weed species was multiplied in the past tens of years, while other species were repressed. These invasive weeds are very competitive and able to decrease yield of crops more often than those were dominant twenty or thirty years ago. However, there is an increasing demand to protect crops using less herbicides and minimize environmental pollution and costs of protection.

Under these circumstances development of more affective methods and reasonable use of them are necessary to control dominant weed species.

For more effective and economical use of several weed control methods it is essential to have knowledge about competitiveness of dominant weeds in the certain area and reasonable degree of weed control.

Ragweed (*Ambrosia artemisiifolia*), jimsonweed *(Datura stramonium*), velvetleaf (*Abutilon theophrasti*), cocklebur species (*Xanthium strumarium, X. italicum*), panic species (*Panicum miliacem, P. capillare*) and some other continuously spreading weed species (Molnár and Précsényi 1996, Szőke 2001, Solymosi 2005) have become dominant species of weed associations of arable lands in Hungary and determine weed controlling processes.

There are a lot of results about competitiveness of these weeds from several parts of the World, but there are not enough datas in Hungary to use them in weed control processes successfully.

Beckett et al. (1988) investigated competition of maize with several weed species. Cockleburs were studied in densities ranging 0.4-6.6 plant/m^2. As result of their investigations it was found that increases in common cocklebur density caused corn yields to decrease curvilinearly in some years, and yields decreased linearly as cocklebur density increased in other

years. They observed 10-27% maximum yield losses at density of 6.6 plant/m^2 in several years. Intraspecific interference among cocklebur plants increased and the individual effect of each additional weed on yield diminished at higher weed densities, so the rate of reduction in maize seed yield declined with increased density.

Bloomberg et al. (1982) studied competition of cocklebur with soybean, and observed that the full season competition of cocklebur at the density of 1 plant/3m of row can reduce the yield of soybean. Soybean seed yield increased at a declining rate as the length of time from soybean emergence to cocklebur emergence increased. They found that when water availability was not unusually limited soybean seed yield was affected most by cocklebur competition, but under conditions of limited water availability soybean vegetative growth was affected most. In their opinion the economic return on the cost of control of cocklebur may depend on water availability during the growing season.

Weber and Staniforth (1957), Cartter and Hartwig (1963) found that common cocklebur plant often form a canopy over the soybean crop while soybean plants are flowering and the resulting shade may increase pod abortion and reduce seed yield.

Barrentine (1974) reported a soybean seed yield reduction of 52% from season-long competition with 26000 cocklebur plants/ha, but removal of weeds within 4 weeks after soybean emergence prevented seed yield reductions. Gossett (1971) observed 50% yield reduction as a result of competition of 14 cocklebur plants/3.1m of soybean row. McWorther and Hartwig (1972) found yield losses ranging from 63 to 75% for six soybean varieties from season-long competition with 7400-16500 cocklebur plants/ha.

Norsworthy (2004) studied cocklebur emergence under soybean and in absence of soybean. Canopy formation (50% light interception) reduce magnitude of daily soil temperature fluctuation from 10-15 ^oC to 5 ^oC compared to absence of soybean at 2.5 cm depth. The red/far red ratio of light available to seed on or near the soil surface was reduced from as much as 1.2 in full sunlight to less than 0.1 in the presence of a dense soybean canopy.

He found that cocklebur emergence diminished after soybean canopy formation, a small portion of its seedbank emerged beneath the canopy. In his opinion its enough to fill up seedbank. It is confirmed by Regnier et al. (1988), who reported a good shade tolerance of cocklebur.

Tranel et al. (2003) call attention to differences of effects of several cocklebur populations. They examined the effect of seven populations from several parts of USA on soybean on the same habitat, which caused yield losses of soybean ranging from 25 to 42%.

Kovács et al. (2006) investigated velvetleaf competition with maize at weed density of 1, 2, 5, 10 plant/m^2. They found that the competition between maize and velvetleaf was not considerable, not even at the highest weed density, because of extreme high amount of rainfall during the vegetation period.
Schweizer and Bridge (1982) studied competition of velvetleaf with sugar beet, and they found 14, 17, 25 and 30% root yield losses at weed density of 6, 12, 18, 24 plants/30m of row, respectively.
Hagood et al. (1980) investigated competition between velvetleaf and soybean at velvetleaf densities ranging from 2.5 to 40 plants/m^2.

Materials and Methods

Field experiments were conducted to study competition of Italian cocklebur, velvetleaf and ragweed with maize and sunflower in experimental sites of the Department of Plant Protection, Debrecen University in 2005 and 2006.
All treatments were established in small plots (25 m^2), in three replications.
Maize and sunflower were seeded in rows spaced 70 cm apart. Plots consisted of seven rows of maize or sunflower, 5 m long. The area of experiments was infected by the studied weed species. The three weed species were hand-thinned 10-15 days after emergence to give densities of 1, 2, 5, 10 plants/m^2 in plots of competition study. These plots were maintained free of other species.
There were established herbicidal weed control experimental plots in which weeds were controlled by one or two herbicidal treatments, and plots which were hoed once, as well.
In 2005 weeds sprouted with crops or in 3-4 days, in 2006 cockleburs and ragweed sprouted with crops but significant part of velvetleaf emerged 10-20 days after maize emergence.
Treatments in maize and sunflower are shown by Tables 1 and 2.
Dominant weed species of the area were ragweed, Italian cocklebur, common lambsquarter, redroot pigweed and velvetleaf.

Table 1. Treatments in maize in 2005 and 2006

	Treatments	2005	2006
1	Weed free control	+	+
2	Weedy control	+	+
3	Hoed at 3-4 leaves stage	+	+
4	Hoed at 6-7 leaves stage	+	+
5	1 plant/m^2	velvetleaf	velvetleaf
6	2 plant/m^2	velvetleaf	velvetleaf
7	5 plant/m^2	velvetleaf	velvetleaf
8	10 plant/m^2	velvetleaf	velvetleaf
9	pendimetalin+dimetenamid(pre) bentazon+dicamba (post)	+	+
10	nicosulphuron + bentazon+ dicamba+ Dash (post)	+	+

Table 2. Treatments in sunflower in 2005 and 2006

	Treatments	2005	2006
1	Weed free control	+	+
2	Weedy control	+	+
3	Hoed at 4 leaves stage	+	+
4	Hoed at 8 leaves stage	+	+
5	1 plant/m^2	velvetleaf	cocklebur ragweed
6	2 plant/m^2	velvetleaf	cocklebur ragweed
7	5 plant/m^2	velvetleaf	cocklebur ragweed
8	10 plant/m^2	velvetleaf	cocklebur ragweed
9	pendimetalin+dimetenamid(pre) imazamox (post)	+	+

Precipitation was more than enough for maize and sunflower during growing season in both years (Figure 1), temperature was optimal for crops and weeds in 2005 but it was below average in the beginning of the season in 2006, and it may effect on emergence of velvetleaf.
Heights of crops were measured in flowering and seed yield was measured after harvest.

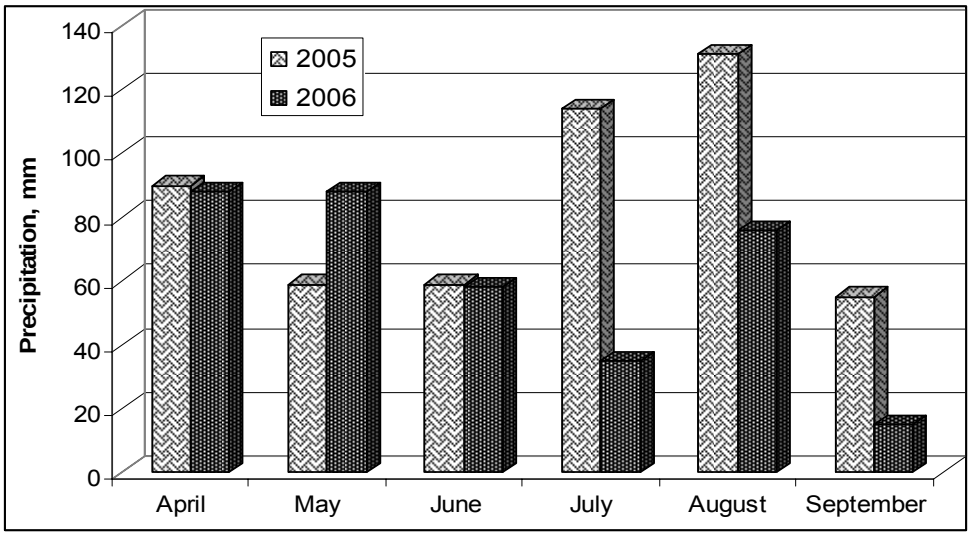

Figure 1. Precipitation on experimental area in 2005 and 2006

Results and Discussion

The height of maize reduced only in weedy control plots compared to weed free control by 12 and 33% in 2005 and 2006, respectively. Other treatments have not significant effects on the height of maize (Figure 2).

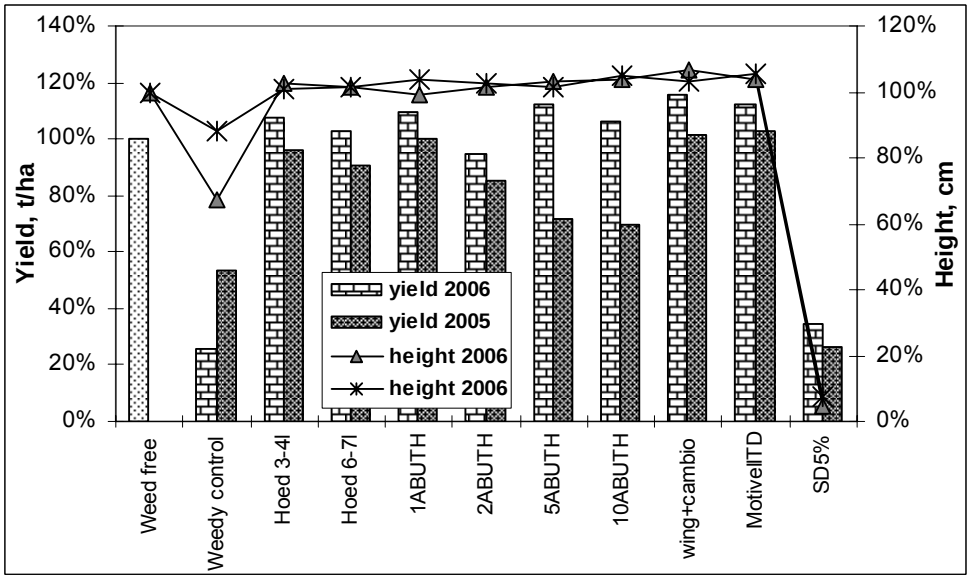

Figure 2. Yield and height of maize in 2005 and 2006

Effects of velvetleaf were significant on seed yield in 2005 in some cases, but it did not reduce yield of maize at any level of competition in 2006.

In 2005, yield was reduced by 47% in weedy control plots. Effects of density of 1 and 2 velvetleaf plants/m^2 were not significant, but 5 and 10 plants/m^2 reduced seed yield by 28 and 31%, respectively. Yield losses could be avoid by hoeing once at 3-4 or 6-7 leaves stages of maize and both of the herbicidal treatments. Good competitiveness of maize and good effects of one-time hoeing in this year may be accounted by plentiful precipitation and optimal temperature, so the growth of maize was very intensive at the beginning of the season when weeds not delayed it that time. In the second part of the growing season weeds were less competitive with the 300 cm height maize.

In 2006, yield was reduced by 74% in weedy control plots, but yields of maize were not influenced significantly in other treatments. One-time hoeing and herbicidal treatments proved seed yield similarly to former year. Most of velvetleaf plants sprouted 10-20 after maize emergence because of below-average temperature in the beginning of the growing season. This may be the main reason that velvetleaf could not reduce yield at any level of competition in this year (Figure 3).

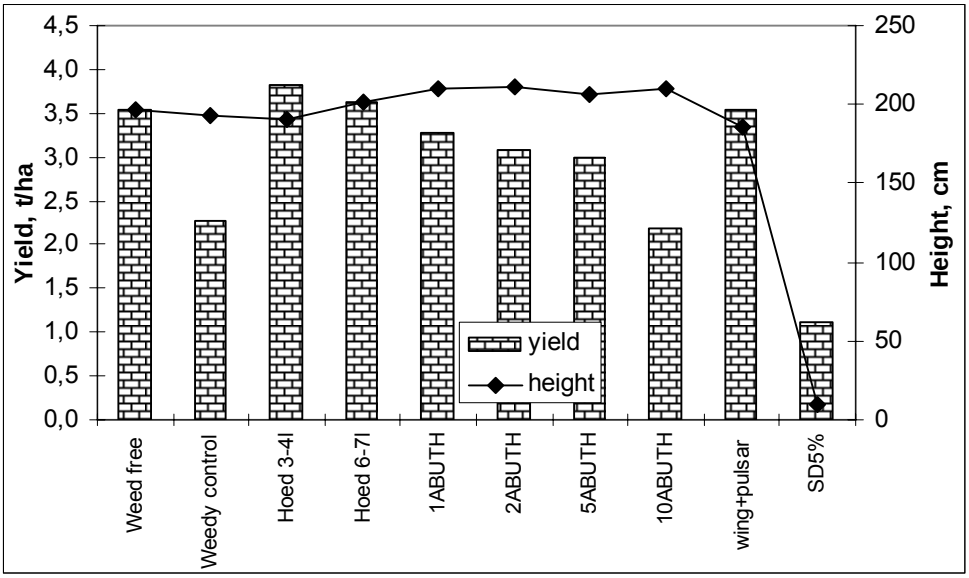

Figure 3. Height and yield of sunflower as results of competition with velvetleaf

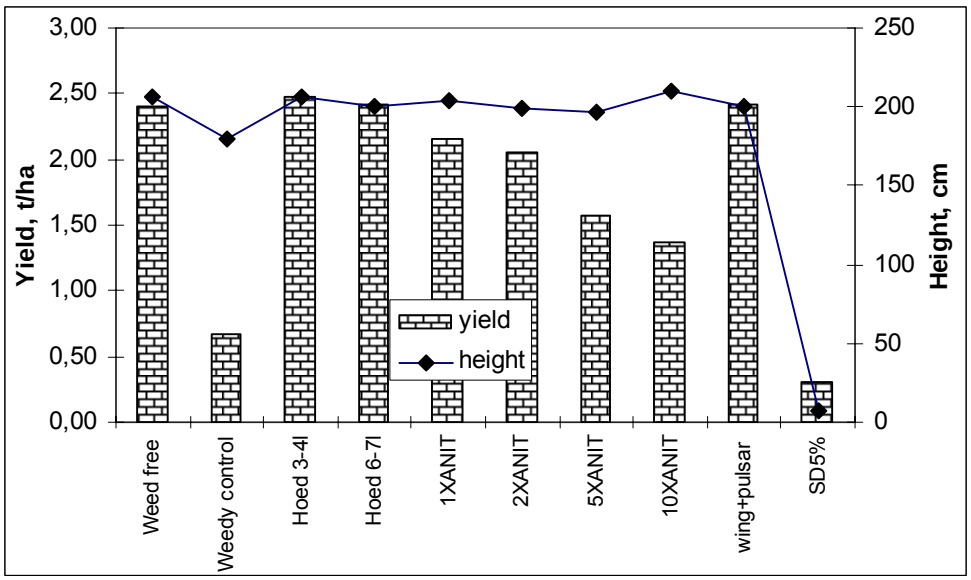

Figure 4. Height and yield of sunflower as results of competition with cocklebur

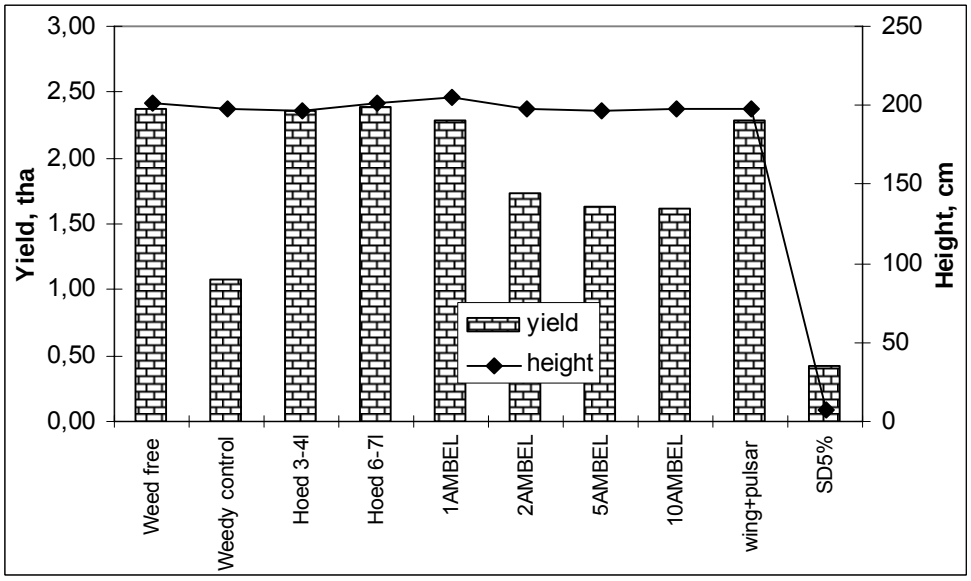

Figure 5. Height and yield of sunflower as results of competition with ragweed

Height of sunflower was not influenced significantly by hoeing and the herbicidal treatment and in weedy control, but sunflower plants elongated by 10-15 cm as results of velvetleaf competition in 2005 (Figure 3).
In 2006, heights of sunflower were reduced only in weedy control of area of cocklebur-competition experiments and by 2 and 5 cocklebur plants/m^2 by

17, 8 and 10 cm, respectively. Other treatments did not influence the height of sunflower (Figures 4 and 5).

In 2005, yield of sunflower were reduced only by 10 velvetleaf plants/m^2 and in weedy control plots up to 62 and 64% of weed free control (*Figure 3*).

In 2006, one-time hoeing and herbicidal treatments proved seed yield of sunflower, and 1 cocklebur or ragweed plants/m^2 did not reduced seed yield. 2, 5 and 10 cocklebur or ragweed plants/m^2 decreased yield by 14, 34, 43 and 27, 31, 32%, respectively. Seed yield of weedy control plots were 28 and 46% of weed free plots on cocklebur-competition and ragweed-competition areas (*Figure 4, 5*).

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COMPETITION OF SUNFLOWER AND MAIZE WITH SEVERAL WEED SPECIES

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Summary

Field experiments were cunducted to sudy competition of maize and sunflower with velvetleaf, Italian cocklebur and ragweed in Deberecen, Hungary in 2005 and 2006. Weed densities were 1, 2, 5 and 10 plants/m^2.

In 2005, velvetleaf emerged with maize reduced seed yield by 28 and 31% at the density of 5 and 10 plants/m^2, respectively. In 2006, velvetleaf emerged 10-20 days after maize did not reduced seed yield at any level of competition. The height of maize was not influenced by velvetleaf competition. In 2005, velvetleaf emerged with sunflower reduced seed yield by 38% at density of 10 plants/m^2, but lower densities of the weed species could not reduce seed yield. In 2006, cocklebur reduced seed yield of sunflower by 14, 34 and 43% at the densities of 2, 5, 10 plants/m^2, respectively, ragweed reduced yield by 27, 31 and 32% at the same densities. The height of sunflower were decreased only by the densities of 2 and 5 cocklebur plants/m^2.

CHANGES IN ALLELOPATHY OF *XANTHIUM ITALICUM* MOR.

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All over the world cocklebur species cause severe problems mainly in row crops, but their damage can be considerable on pasturelands and as invasive species in natural communities as well. Therefore, several authors studied the biology, competitiveness and the regulation potentials of the *Xanthium* genus.

Besides the widespread examination of competition ability, a relatively less intensively studied area is the allelopathy of cocklebur, although its existence has been identified for a long time. Therefore, the role of allelopathy in the competitiveness of the species has not yet been clarified. The actual role of allelopathy is controversial not only in cockleburs, but in other allelopathic weed species as well. The reason for this is the fact that allelopathy is influenced by several environmental factors and their heterogenicity affected research results as well.

Rice (1964) is one of the first investigators studying allelopaty of cockleburs. He studied the effect of *Xanthium* spp. and other species on nitrogen-fixing and nitrifying bacteria (*Azotobacter, Rhizobium, Nitrobacter, Nitrosomonas*).

Extracts of cockleburs inhibited several *Azotobacter, Rhizobium, Nitrobacter* strains. He found also that effect of cocklebur samples are different depending on collecting dates, however, he did not observe differences in phenological stages of plants. He hypothetised that environmental factors modified the allelopathy.

He found inhibitory effect not only in case of extracts but in case of soil of cockleburs.

Rice also observed that effects of extracts depended on the age of the plat organs were used for tests.

Bushra et al. (1987) studied effects of extracts of several organs of cocklebur on germination and growth of lettuce, *Brassica campestris*, maize and *Pennisetum americanum*. Both residues and leachates of plants were effective in their experiments. They determined caffeic acid, p-hidroxi-benzoic acid, p-coumaric acid, klorogenic acid as allelopathic agents.

Einhelling et al. (1985) examined the effects of allelochemicals and allelopatic plants (common cocklebur) on plant-water relationships.

Cinnamic acid derivatives increased leaf diffuse resistance, decreased water potential, osmotic potential and dried weight of grain sorghum. Cocklebur

residues decreased only dried weight significantly compared to the untreated plants.
Chon et al.(2003) studied extracts of 16 *Compositae* plants. The results showed the highest inhibition for the extracts from *Lactuca sativa*, *Xanthium occidentale* and *Cirsium japonicum*. They identified trans-cinnamic acid, chlorogenic acid, coumarin, coumaric-acid from extracts of cocklebur.
Casini (2004) examined extracts and residues of cockleburs on maize. He found strong inhibitory effect of either extracts or residues, but his results differ from those observed by Bushra et al. (1987).
He examined several maize hybrids, and found differences among sensitivity of them against allelopathy of cocklebur.
Cutler and Cole (1983) studied allelopathic affects of atractylozide and carboxyatractylozide. These compounds can be found in young cocklebur plants, and cause poisoning mainly in sheep, cattles and pigs. They observed inhibition and necrotic symptoms on maize, wheet and tobacco.

Materials and Methods

Study on allelopathy in biotests

The allelopathy of Italian cocklebur was studied in several biotests from 2002 to 2005, for which we used fresh and dried (at 60 ^{o}C for 48 hours) weed sprouts and roots separately. We used the remnants of sprouts and roots separately and in a mixture as well. During the preparation of extracts, for 100 ml of solvent we used fresh sprout or root of 4, 8, 16, 20g, or the respective amount of dried parts.
The applied solvent was tap water or ethanol (96%). Extraction took place at room temperature for 24 hours in darkness. The test plants used in biotests were garden cress (*Lepidium sativum* L.), sugar beet (*Beta vulgaris* L.), spring barley (*Hordeum distichon* L.) and maize (*Zea mays* L.), which were germinated in Petri-dishes (diameter: 11cm). We evaluated the germination, root and sprout growth of sugar beet, as well as the root and sprout growth of the other three test crops.
We examined the following influences of factors affecting allelopathy: the means of lighting during donor plant production, the development stage of donor plants, the density and water supply of their population, the effect of precipitation, the preparation of samples for extraction (using fresh or dried and ground plants) and the type of the extracting agent.

Besides germination trials, we followed-up the quantitative changes in cinnamic acid derivatives, which are known as allelochemicals detected earlier from cockleburs, i.e. chlorogenic acid, coumarin, p-coumaric acid and trans-cinnamic acid.
For the quantitative determination of the 4 compounds we prepared an extract from dried crop samples with distilled water, using a sample of 4 g and distilled water of 100 cm^3, which was shaken for 2 hours. After filtering, the agents were identified with Merck-Hitachi HPLC equipment. The circumstances of separation were the following:
column: Lichrospher 100RP-18, 125x4mm;
12:15:1 mixture of eluent: water: methanol: acetic acid;
flow: 1ml/minute.
Detecting was performed with a L-4500 Diode Array Detector at a wave length of 275 nm. For quality identification we used a comparative solvent liquid containing chlorogenic acid (SIGMA), p-coumaric acid (SIGMA), coumarin (SIGMA) and trans-cinnamic acid (ALDRICH).
The quantitative identification of allelochemicals took place in the Regional Agro-Instrument Centre, Centre of Agricultural Sciences, University of Debrecen.
The gained values were compared with the findings of biotests.

Results and Discussion

We tested the allelopathy of Italian cockleburs grown in fields and in greenhouses on cress, maize and spring barley.
Extracts prepared from the fresh and dry parts, the root and sprout remnants of Italian cocklebur affected the test plants and their vital processes (germination, growth) in a different way; however, all test crops verified the allelopathic effect in the performed biotests. Treatments affected the germination and growth of sugar beet, whereas they influenced only the growth of the other three test crops.
However, the rate and means of allelopathy depended not only on the species of the test crop, but on other factors as well, which supposedly influence the production, secretion and solubility of allelochemicals.

- The way and rate of the effect changed depending on the used plant organs.
- Biotest results were determined by the fact, whether extracts were prepared from live plants or from plant remnants.
- The effect was influenced by the solvent (water, ethanol) on certain test crops.

- Extracts prepared from the same parts of plants grown in fields and in greenhouses resulted in different effects.
- The inhibitory effects of extracts prepared from cockleburs grown in greenhouses also depended on the fact, whether the weeds were produced in short or long day circumstances.
- The effects of cocklebur grown in fields were affected by the water supply of the weeds before sample taking, by the amount of precipitation before sample taking, the phenological stage of the weeds and the density of their population.
- The primary material of samples (fresh, dried and ground) also affected allelopathy.
- Germination temperature also influenced the end result of biotests.

The effects of extracts prepared from fresh plant parts for test crops can be summarized in the following way:

Italian cocklebur extracts inhibited the germination of *sugar beet* test crops in a smaller or greater level, however, factors mentioned earlier affected the power of the inhibitory effect and the rate of effect loss. In general, it can be concluded that the effect of sprout extracts was greater than that of root extracts. In 2003 the germination inhibiting effect of young plant extracts was stronger than that of blooming plants, whereas such difference could not be detected in 2004. As a result of heavier precipitation, extracts had a greater germination inhibiting effect in both years. Out of the two years, this effect of precipitation was less significant in 2004, in which year the weather was more favourable and received more balanced precipitation.

As long as extracts were effective, they affected the growth of the test crops positively, but in some cases inhibitory effects also emerged. Extracts were more effective on the root growth of the crops than on their sprout growth.

In the case of *cress*, only some extracts of great concentration could cause the large-scale inhibition of germination. However, their effects on growth varied on a large scale in relation to the antecedents and circumstances of extract preparation. Regarding the crop parts used for extract preparation we experienced that sprout extracts were mostly of greater inhibitory effect than root extracts. In sprout extracts, the inhibitory effects exercised on test crops were greater before precipitation than after it, when in several cases inhibition did not take place or the extracts had stimulating effects.

In root extracts this phenomenon could not be observed; moreover, several cases showed exactly its opposite. However, the effect was influenced not only by precipitation, but by other factors as well. The growth of garden cress was inhibited by the extracts of blooming plants more significantly in both years, than those of 4-5 leaf plants. Nevertheless, the effect of cocklebur population density was only significant in the case of some

samples in 2004. Extracts affected the sprout growth of test plants less, than their root growth.

Maize was usually less sensitive to the same extracts than the above mentioned two test crops, but precipitation, population density and the phenology of donor plants influenced allelopathy in this case as well. Extracts from cockleburs grown in fields inhibited maize growth solely in the case of root samples from dense populations in July, but after precipitation this effect could not be experienced.

In the case of *cress* and *sugar beet* we compared the effects of extracts from fresh crop parts with the effect of extracts from the dried and ground pieces of the samples. In the case of cress the effects of extracts from fresh and dried parts were similar (on the 6th day: R=0.709), but the latter was more effective (Figure 1). However, the composition and solubility of allelochemicals effecting on sugar beet modified in the course of drying and grinding so much that there was no correlation between the effects of extracts from fresh and dry samples on day 10th in respect of germination R=0.049, and root growth R=-0.262 (Figure 2).

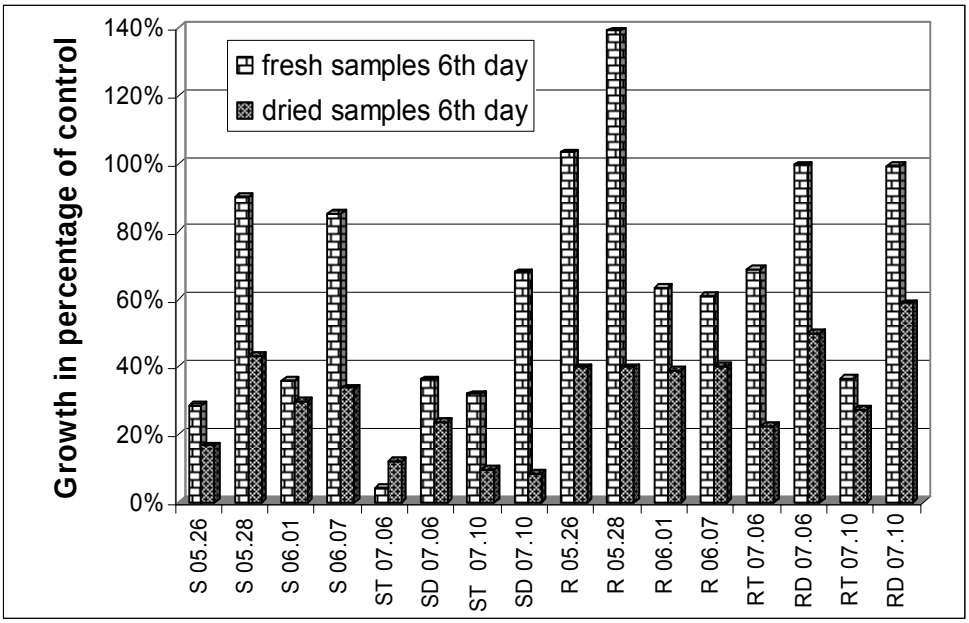

Figure 1. Effects of extracts made from fresh and dried samples of cocklebur on root growth of cress at 6th day

S: shoot extract; R: root extract; T: extract made from thin stand of cocklebur; D: extract made from dense stand of cocklebur.

(Concentration of extract made from fresh cocklebur is 12g/100ml, made from dried cocklebur is equivalent to the fresh cocklebur extract.)

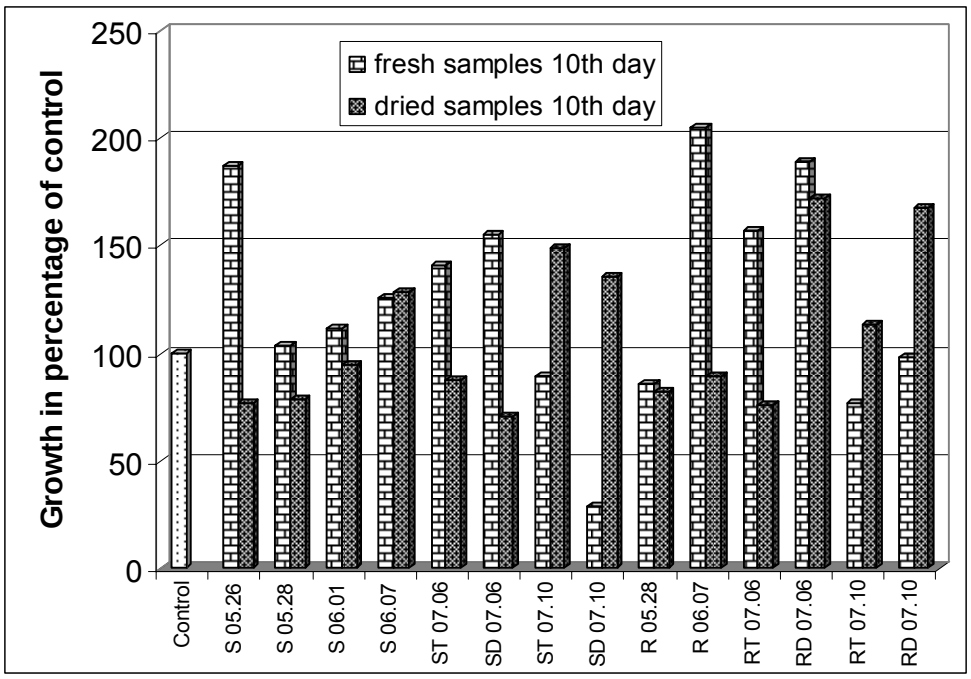

Figure 1. Effects of extracts made from fresh and dried samples of cocklebur on root growth of sugar beet at 10^{th} day

S: shoot extract; R: root extract; T: extract made from thin stand of cocklebur; D: extract made from dense stand of cocklebur.

(Concentration of extract made from fresh cocklebur is 12g/100ml, made from dried cocklebur is equivalent to the fresh cocklebur extract.)

The effects of cocklebur remnants on test crops were determined by the temperature of germination. Inhibitory effects on test crops were more significant at a lower temperature, which was less favourable for germination. The growth of cress was inhibited by extracts in a greater extent at a lower temperature than at room temperature.

In fact, extracts could not inhibit the germination of sugar beet at room temperature, but at 8-10 ^{o}C it was inhibited by each extract containing root remnants. However, there was no significant difference in the stimulating effect on sugar beet growth, depending on the temperature of germination. Maize proved to be sensitive to extracts containing sprout remnants at temperatures unfavourable for germination; however, at room temperature all extracts were ineffective for this test crop.

In the sprouts and roots of cockleburs growing under natural field conditions, as a result of the changeability of allelopathy, we could follow-up the quantitative changes of four allelochemicals: that of trans-cinnamic acid, coumarin, p-coumaric acid and chlorogenic acid. The concentration of

the given compounds showed a modification of 4.6-15.5 times in sprouts, and that of 2.6-29.8 times in roots in the vegetation period (Figures 3 and 4).

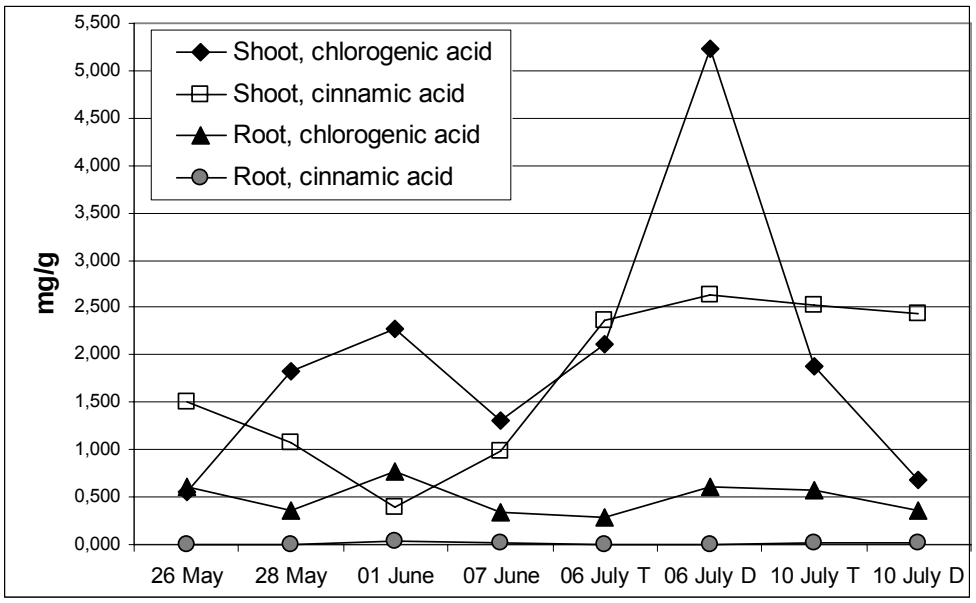

Figure 3. Changes in amounts of chlorogenic acis and trans-cinnamic acid in shoots and roots of cocklebur

T: extract made from thin stand of cocklebur; D: extract made from dense stand of cocklebur

Although allelopathy is mostly influenced by the joint effects of several compounds, the quantitative changes of p-coumarin acid and trans-cinnamic acid showed a correlation with the effects of extracts on cress. The correlation between the quantity of p-coumaric acid and the early sprout growth of cress was: $y=-7.0535+(-3.3198*\ln x)$ ($R^2=0.811$); the correlation with root growth was: $y=-11.009+(-5.7628*\ln x)$ ($R^2=0.775$).

The quantitative changes of cinnamic acid showed a correlation with the root growth of cress, which was experienced at a later measurement ($R=-0,743$). However, in other cases we could not draw conclusions from the quantitative changes of any compounds on the growth and germinating power of the test crops, which denoted that the effects of other allelochemicals in cockleburs might be significant.

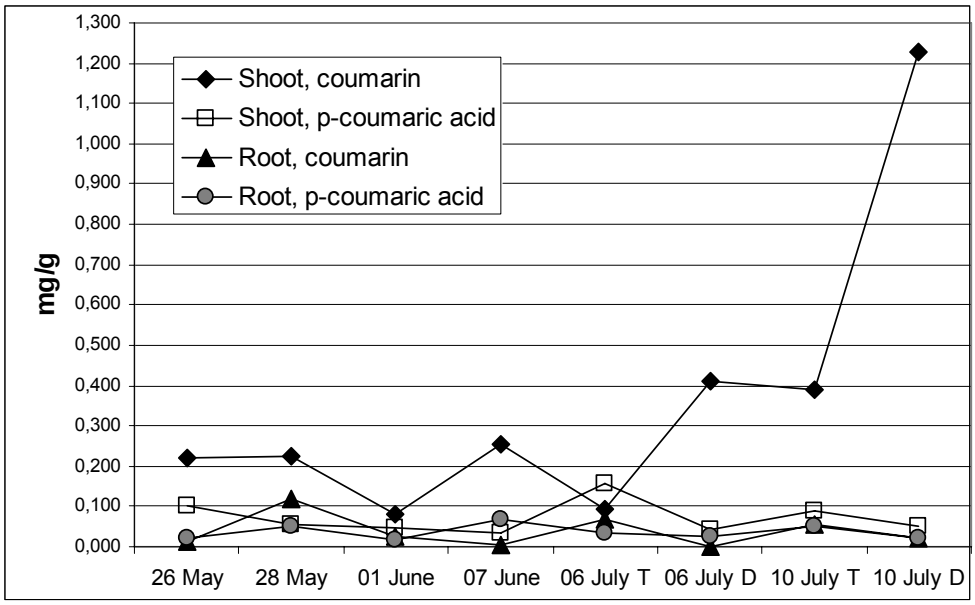

Figure 4. Changes in amounts of coumarin and p-coumaric acid in shoots and roots of cocklebur

T: extract made from thin stand of cocklebur; D: extract made from dense stand of cocklebur

The changeability of allelopathy caused by cockleburs, which can be experienced on test crops and the significant quantitative differences of allelochemicals depending on external and internal factors make it necessary to find out the factors which are responsible for the changes, to quantify their effects and to take them into consideration in studying allelopathy so that we can use research findings more extensively.

On the basis of our findings, the roles of the studied and other factors can be significant in many cases. Further investigations are needed to find out the effective factors in certain cases of allelopathy and to identify their exact effects. The factors which mostly influence research findings are to be identified, e.g. from the quantity and intensity of precipitation before sample taking, we can only draw direct conclusions concerning the extraction of allelochemicals from crop sprouts. Crop water supply and its changes are caused, besides the effects of occasional precipitation, by the water supply capacity of soil before and after rain, atmospheric dryness etc., so considering these facts, the effect of precipitation can only be considered indirect. In these cases, the measurement of qualities which reflect crop water supply better, such as water potential could be suggested for further examinations.

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CHANGES IN ALLELOPATHY OF *XANTHIUM ITALICUM* MOR.

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Summary

Cockleburs are noxious weeds in the World and in Hungary, as well. Its allelopathy is one of the reason that it was examined in several studies. Extract's and residues of the weed were found to be effective against crops and other weeds, and some chemicals were determined which can play role in it. In some cases results of investigation of allelopathy don't reproducible exactly. The reason is, that there are some factors wich can modify the outcome of allelopathy but these are not taken into consideration. Several biotest were conducted to study modifying factors of allelopathy, and some allelochemicals were quantified in relation with these factors. The way and rate of the effect changed depending on the used plant organs. Biotest results were determined by the fact, whether extracts were prepared from live plants or from plant remnants. The effect was influenced by the solvent (water, ethanol) on certain test crops. Extracts prepared from the same parts of plants grown in fields and in greenhouses resulted in different effects. The inhibitory effects of extracts prepared from cockleburs grown in greenhouses also depended on the fact, whether the weeds were produced in short or long day circumstances. The effects of cocklebur grown in fields were affected by the water supply of the weeds before sample taking, by the amount of precipitation before sample taking, the phenological stage of the weeds and the density of their population. The primary material of samples (fresh, dried and ground) also affected allelopathy. Germination temperature also influenced the end result of biotests. The concentration of the four compounds (trans-cinnamic acid, coumarin, p-coumaric acid and chlorogenic acid) showed a modification of 4.6-15.5 times in sprouts, and that of 2.6-29.8 times in roots in the vegetation period. Although allelopathy is mostly influenced by the joint effects of several compounds, the quantitative changes of p-coumarin acid and trans-cinnamic acid showed a correlation with the effects of extracts on cress.

POSTER SESSION

EFFECT OF TEMPERATURE ON THE GROWTH OF *MACROPHOMINA PHASEOLINA* ISOLATES

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The economic importance of charcoal rot disease caused by *Macrophomina phaseolina* (Tassi) Goidanich [synanamorf: *Rhizoctonia bataticola* (Taubenhaus) E.J. Butler] is still considerable. This polyphagous pathogen infects more than 300 plant species. In Hungary it causes serious damage – especially in dry, hot seasons – on sunflower, maize, legumes, paprika and many other plants (Békési, 1970; Varga et al., 1997). The damage influenced mainly by the season, location, water and nutrient supply. The disease can be diagnosed on the basis of the symptoms: ash grey spots on the stems and small, black microsclerotia developed in the pith and root tissues. The effect of temperature on the growth of *Macrophomina* has been investigated earlier by Das (1988), who found that the optimal temperature of for mycelia growth and microsclerotia development was 30 °C in India.
The aim of our study was to investigate the effect of temperature on growing pattern of 35 *Macrophomina phaseolina* isolates from different Hungarian locations.

Materials and Methods

The experiments have been made under the same conditions for all *Macrophomina phaseolina* isolates, except the different temperatures, where the growing pattern of the fungal colonies were measured. Initial test-material was collected from 32 different sunflower fields in September, 2005 (Table 1 and 2). From one site (Cserkeszőlő), two samples were collected, one from cultivated sunflower and the other one from a volunteer sunflower. From three sites (Bóly, Iregszemcse, Keszthely) samples were collected from soybeans, too. Scrapings from the infected plant debris were taken to potato dextrose agar (PDA) medium, at 25 °C. Pure cultures were made by threefold passage. PDA medium were poured into 9 cm diameter sterilized Petri dishes. 5 mm in diameter agar discs with overwintering propagules were taken from the well growing cultures kept on 25 °C and transferred to agar media. Following the inoculation Petri dishes were taken in darkness into thermostats adjusted to 10, 15, 20, 25, 30, 35 and 40 °C, respectively. The effect of temperature on growing patterns of *Macrophomina* was tested in four replications. Colony diameters were

measured after 3, 5 and 6 days followed the inoculations, respectively. Statistical analysis was made by Microsoft Excel programme. Because of the large extent of the samples this work shows only representative results.

Results

It is typical for this pathogen, that first the mycelia start to growth and then follows the formation of microsclerotia. The heat tolerance of isolates showed a wide range of differences. The average of mycelium colonies in diameters are given in the Table 1, and the diameter of microsclerotial colonies are summarized in the Table 2. Considerable differences among the isolates can be seen in both cases. Significance values were given on LSD for 5 % probability.

At 10 °C no mycelia growth was observed, therefore no data are present in the tables. Even on the 5th day some of the isolates showed a small mycelium growth, at 10 °C was very slow one, the highest daily growth rate was only 0.71 mm/day at soybean isolate (Iregszemcse).

At the 3rd day at 15 °C only two isolates started to grow, one from sunflower (Tordas) and the other one from soybean (Iregszemcse). In the average of four replication the growing rate of Tordas isolate was only 1.0 mm/day, and that of Iregszemcse was 4 mm (value of SD 5% was 0.33). The 5th day slow growing rates were observed in nine isolates of all ones (Bize, Dunaföldvár, Hódmezővásárhely, Kaposvár-Toponár, Keszthely, Lepsény, Nyíregyháza, Szederkény and Székkutas) from sunflower, and one (Keszthely) from soybean. On the 6th day seven other isolates started their mycelial growth. At 15 °C the Iregszemcse isolate showed the highest average daily growth rate (2.04 mm).

Because of the lack of microsclerotia formation on the 3rd day at 10, 15, 20 and 40 °C, and on the 5th and 6th day 10, 15, and 40 °C, data are not included in the Table 2. The Table 1 does not show the values of the 5th and 6th days mycelium colony in diameters at 25, 30 and 35 °C because all have reached the sides of Petri dishes (90 mm), except the soybean isolate of Iregszemcse. This value on the 5th day at 25 °C was 45.75 mm, at 30 °C was 47.25 mm, and at 35 °C was 41.75 mm. On the 6th day at 25 °C the diameter of the colonies was 54.25 mm, at 30 °C 56.50 mm, and at 35 °C 46.0 mm, respectively, that means, that this isolate significantly differed from the other isolates. The Table 2 does not show the values of microsclerotial diameters at 25, 30 and 35 °C, at the 5th and 6th days because these isolates have already reached a value of 90 mm, except the soybean isolate of Iregszemcse. The Iregszemcse isolate showed a microsclerotial value on the 5th day at 25 °C was 31.25 mm; at 30 °C 33.50 mm; at 35 °C 32.0 mm. On the 6th day at 25 °C was 42.00 mm; at 30 °C

47.25 mm; at 35 °C 35.0 mm. This isolate significantly differed from the other ones.

Table 1. Average colony growing in diameters of *Macrophomina* isolates

Site of collection	Colony in diameters (mm)						
	3rd day					5th day	
	20	25	30	35	40	10	15
Balatonújlak	30.00	72.00	90.00	90.00	1.75	2.00	2.00
Bize	26.25	78.50	87.00	89.75	31.25	1.5.	1.50
Boda	29.00	79.50	89.25	90.00	15.25	0.00	0.00
Bóly	26.75	78.25	90.00	90.00	10.25	0.00	0.00
Böhönye	33.25	67.75	74.75	90.00	1.25	1.50	2.00
Cserkeszőlő	26.00	69.25	74.75	89.00	3.25	0.00	0.00
Cserkeszőlő*	20.75	55.75	76.00	71.25	2.00	0.00	0.00
Debrecen	17.75	62.75	72.50	80.25	0.00	0.00	0.00
Dunaföldvár	28.75	69.25	76.25	89.25	11.00	0.00	1.50
Gyulafirátót	30.00	68.25	79.00	76.00	4.00	1.50	1.75
Hódmezővásárhely	24.25	61.75	69.00	82.75	0.75	0.00	2.00
Kadarkút	29.00	76.25	87.00	87.75	7.00	0.00	0.00
Kaposvár-Toponár	30.00	77.50	89.25	90.00	21.75	0.00	0.75
Karcag	17.25	63.25	76.75	75.75	1.25	1.75	1.00
Kecskemét	38.75	64.50	90.00	90.00	0.00	0.00	0.00
Keszthely	30.25	65.75	81.00	90.00	0.00	0.00	1.75
Kéthely	29.00	69.50	81.00	89.00	62.25	0.00	0.00
Kunszentmárton	28.25	66.50	75.75	90.00	9.00	0.00	0.00
Lakitelek	29.00	70.00	88.00	78.75	0.00	0.00	0.00
Lepsény	28.75	77.75	90.00	90.00	2.00	0.00	2.00
Mesztegnyő	29.25	84.00	89.25	90.00	0.25	1.50	1.25
Nagykanizsa	27.00	61.25	85.00	80.00	0.00	0.00	0.00
Nyíregyháza	20.00	61.00	69.75	75.50	0.25	0.00	0.50
Pogányszentpéter	28.25	64.00	88.75	87.00	4.75	0.00	0.00
Röjtökmuzsaj	18.00	64.25	73.75	72.75	0.00	1.50	1.00
Sármellék	32.25	80.25	81.75	90.00	25.25	0.00	0.00
Szederkény	27.25	66.00	68.25	83.00	2.75	0.00	1.75
Szentes	19.75	65.50	81.75	90.00	1.00	0.00	0.00
Székkutas	30.00	60.00	66.00	73.00	2.00	0.00	1.75
Szigetvár	27.00	85.25	83.25	88.00	1.00	0.00	0.00
Tiszakürt	22.25	69.50	82.25	90.00	0.25	0.00	0.00
Tordas	28.75	59.25	65.75	70.25	0.00	0.00	2.50
Bóly **	18.00	52.50	82.25	71.75	0.00	0.00	0.00
Iregszemcse **	13.75	31.00	29.00	29.50	0.00	3.75	8.75
Keszthely **	23.75	70.00	89.25	81.50	0.00	0.00	0.75
Average	**26.24**	**67.65**	**79.24**	**82.62**	**6.33**	**0.43**	**0.99**
LSD 5%	**2.51**	**4.78**	**5.09**	**5.07**	**3.38**	**0.65**	**0.93**

* volunteer sunflower, ** soybean

Table 1. (continued) Average colony growing in diameters of *Macrophomina* isolates

Site of collection	Colony in diameters (mm)					
	5th day		6th day			
	20 °C	40 °C	10 °C	15 °C	20 °C	40 °C
Balatonújlak	73.00	2.00	2.25	2.25	90.00	6.00
Bize	70.25	37.00	1.50	3.00	90.00	39.75
Boda	71.00	29.75	0.00	2.75	88.75	31.75
Bóly	72.00	35.75	0.00	1.50	90.00	37.75
Böhönye	72.25	24.75	2.25	2.25	90.00	27.25
Cserkeszőlő	69.75	4.00	0.00	0.00	90.00	13.75
Cserkeszőlő *	53.75	6.25	0.00	0.00	71.00	22.25
Debrecen	52.25	13.25	0.00	1.00	80.75	24.75
Dunaföldvár	68.75	15.25	0.00	3.25	88.75	17.00
Gyulafirátót	66.00	19.00	2.75	3.50	90.00	21.25
Hódmezővásárhely	59.25	16.75	0.00	4.50	89.00	18.75
Kadarkút	64.00	12.75	0.00	1.00	90.00	17.25
Kaposvár-Toponár	73.25	25.25	0.00	1.25	90.00	30.25
Karcag	55.75	16.00	2.25	1.50	86.00	18.25
Kecskemét	78.75	2.25	0.00	0.00	90.00	8.25
Keszthely	73.25	18.75	0.00	3.25	90.00	32.00
Kéthely	63.75	66.25	0.00	0.00	86.00	68.75
Kunszentmárton	66.25	11.25	0.00	0.00	80.00	14.00
Lakitelek	74.00	3.75	0.00	0.00	90.00	14.75
Lepsény	75.00	3.00	0.00	5.25	90.00	4.25
Mesztegnyő	80.00	4.25	1.75	1.50	90.00	7.00
Nagykanizsa	71.00	2.75	0.00	0.00	90.00	5.25
Nyíregyháza	62.25	3.00	0.00	1.50	90.00	9.25
Pogányszentpéter	68.25	40.25	0.00	1.25	86.75	43.25
Röjtökmuzsaj	41.00	0.00	1.75	2.25	51.75	0.00
Sármellék	71.75	41.25	0.00	1.75	90.00	53.25
Szederkény	63.75	20.00	0.00	3.00	88.25	22.75
Szentes	55.75	12.25	0.00	0.00	72.00	14.00
Székkutas	68.75	16.25	0.00	5.25	90.00	19.25
Szigetvár	73.25	41.75	0.00	0.00	90.00	57.25
Tiszakürt	59.75	7.25	0.00	0.00	75.75	12.75
Tordas	67.00	0.00	0.00	4.25	85.25	0.00
Bóly **	57.25	0.00	0.00	2.25	76.00	0.00
Iregszemcse **	25.00	0.00	4.25	12.25	33.25	0.00
Keszthely **	56.75	4.75	0.00	1.75	85.00	11.75
Average	**64.96**	**15.91**	**0.54**	**2.09**	**84.12**	**20.68**
LSD 5%	**3.96**	**8.23**	**0.68**	**0.71**	**3.70**	**9.07**

* volunteer sunflower ** soybean

Table 2. Average microsclerotial colony growing in diameters of *Macrophomina* isolates

Site of collection	Colony in diameters (mm)				
	3rd day			5th day	6th day
	25 °C	30 °C	35 °C	20 °C	20 °C
Balatonújlak	51.00	89.00	77.25	52.00	80.00
Bize	58.50	70.25	88.25	48.00	76.00
Boda	61.75	80.00	82.25	47.75	74.25
Bóly	59.00	88.75	88.25	46.75	77.25
Böhönye	54.00	65.00	85.75	49.25	79.75
Cserkeszőlő	49.50	56.75	56.00	41.75	71.00
Cserkeszőlő *	42.50	60.75	56.25	35.00	58.00
Debrecen	48.25	54.00	61.00	18.25	51.75
Dunaföldvár	55.75	64.25	63.00	48.00	77.75
Gyulafirátót	51.75	64.25	66.25	50.00	75.00
Hódmezővásárhely	45.50	57.25	63.75	38.75	61.25
Kadarkút	56.25	70.75	79.00	42.75	72.25
Kaposvár-Toponár	61.00	88.25	86.00	52.00	78.25
Karcag	45.00	63.75	63.00	35.25	59.75
Kecskemét	50.00	82.50	88.25	29.75	68.25
Keszthely	46.75	68.00	67.00	50.00	79.75
Kéthely	47.50	73.00	86.25	43.00	71.00
Kunszentmárton	48.75	59.75	67.25	40.00	60.75
Lakitelek	49.75	81.00	76.25	50.75	81.75
Lepsény	57.25	73.25	90.00	48.25	85.25
Mesztegnyő	61.75	82.00	88.00	50.25	85.00
Nagykanizsa	43.00	73.25	59.75	46.00	75.75
Nyíregyháza	49.25	51.25	61.00	16.25	62.50
Pogányszentpéter	44.75	75.25	77.75	44.75	63.25
Röjtökmuzsaj	45.50	52.75	55.75	29.75	44.00
Sármellék	65.25	69.25	86.00	50.00	78.75
Szederkény	51.25	55.75	59.75	42.00	67.00
Szentes	50.00	62.75	67.25	35.00	58.25
Székkutas	45.50	53.00	61.75	48.00	76.25
Szigetvár	60.75	74.25	63.00	51.25	83.00
Tiszakürt	51.25	63.25	85.25	30.25	63.75
Tordas	42.25	53.75	59.00	45.25	69.25
Bóly **	37.00	68.00	64.50	41.25	64.75
Iregszemcse **	13.25	16.25	17.00	14.00	23.00
Keszthely **	50.00	87.75	65.25	42.25	62.75
Average	**50.01**	**67.11**	**70.34**	**41.53**	**69.04**
LSD 5%	**4.19**	**5.67**	**9.02**	**4.31**	**6.92**

* volunteer sunflower ** soybean

On the 3rd day the Kecskemét isolate had the highest growing rate (38.75 mm in diameter). Most of the isolates showed a full (90 mm diameter) mycelium size 5th day after inoculation. It means a growth rate of 15 mm/day. First microsclerotia formation was observed at the 5th day at 20 °C. It is typical that even at the 6th day the microsclerotia did not reach the side of the Petri dishes. The sunflower isolate from Lepsény showed the largest diameter (85.25 mm) with a daily growth rate of 14.21 mm.
On the 3rd day the Szigetvár sunflower isolate showed the largest mycelium colony in diameter (85.25 mm) at 25 °C, while the largest microsclerotial diameter (65.25 mm) was observed by the sunflower isolate of Sármellék.
Mycelium colony diameter of the isolates of Balatonújlak, Bóly, Kecskemét and Lepsény from sunflower at 30 °C reached the maximal diameter on the 3rd day. The microsclerotial diameter of the isolate from Balatonújlak showed just one mm smaller value. The Iregszemcse isolate from soybean showed the slowest growth both at 20 and 25 °C. Except this isolate mycelia and microsclerotial colony diameters of all reached the side of Petri dishes on the 5th day (90 mm), and the same growth was observed at 30 and 35 °C too.
As it is shown in the Table 1, the 3rd day at 35 °C colonies of thirteen isolates from sunflower reached the side of the Petri dishes. At this temperature, as well as at 30 °C, the Tordas isolate showed the slowest growing rate (70.25 mm diameter).

Ten isolates showed no mycelia growth at 40 °C on the 3rd day. Isolates of Röjtökmuzsaj and Tordas from sunflower, and the isolate of Iregszemcse from soybean did not start growth neither on the 5th nor on 6th day. A wide growth interval could be observed on the 6th day. The largest diameter had the isolate of Kéthely (68.75 mm), and lowest daily growth rate was observed by the isolate of Lepsény (0.71 mm/day).
It is interesting to note that while the isolate of Iregszemcse from soybean had the best growing at 10 and 15 °C, it could not grow larger as 60 mm at 20, 25, 30 and 35 °C at a temperature range which all other isolates reached 90 mm mycelium in diameter. Also its microsclerotial diameter was about 50 % of the others even on the 6th day. This difference can be seen well on Figure 1. Upwards of the figure (A) is the Szigetvár isolate from sunflower, and at the bottom the Iregszemcse isolate from soybean, left at 30 °C (B), and right at 35 °C (C).

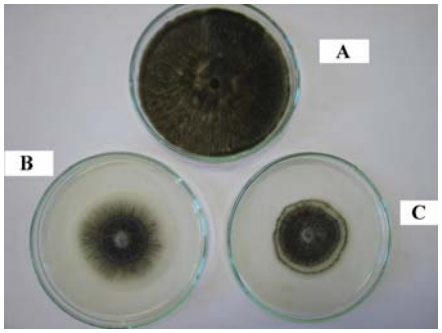

Figure 1. Macrophomina colonies at the 6th day. Isolate from Szigetvár (A) at 35 C°, Iregszemcse at 30 °C (B), and at 35 °C (C)

The Figure 2 shows the average colony diameters of 35 isolates measured at different temperatures on the 3rd, 5th and 6th day.

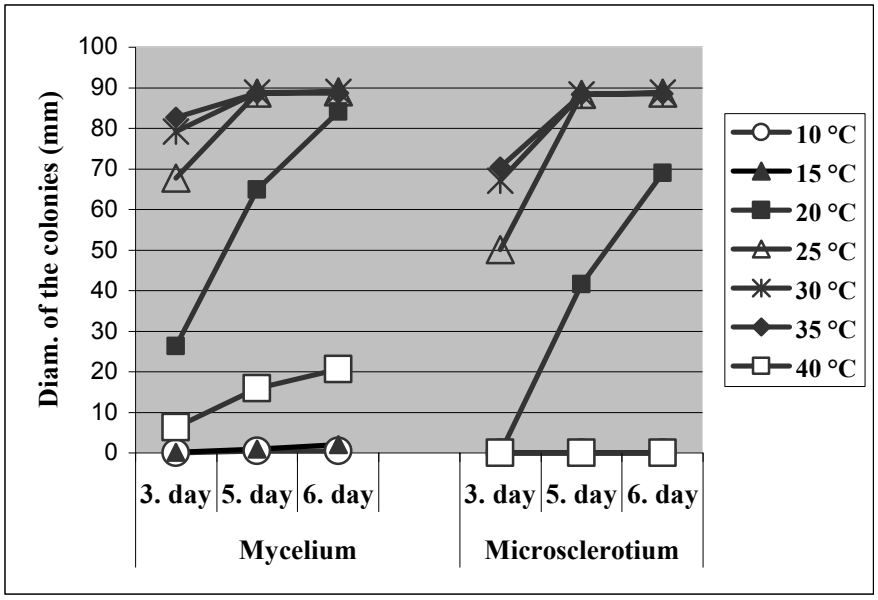

Figure 2. Mycelial (A) and microsclerotial (B) colony in diameters depending on the temperature and incubation time

The most favourable temperature regime for the development of the isolates were between 25 to 35 °C. At 20 °C the isolates were growing relatively well. However the low temperatures as 10 and the high 40 °C was not lethal for the isolates: giving back them to 25 °C, they started to grow.

Discussion

Effect of temperature on the growth of 35 *Macrophomina* isolates was studied. Their growth at 10 and 15 °C was very slow, the average daily growing rate was only 0.71 and 2.04 mm/day, respectively. *Macrophomina phaseolina* did not developed microsclerotia at the too low and at too high temperatures, however at 20 °C on the 5th day they did. The most favourable temperature regime was between 25 and 35 °C for all the isolates. On the 5th day at favourable temperatures all the isolates grown well. Microsclerotial colonies reached the 90 mm in diameter, except for one isolate (Iregszemcse).

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EFFECT OF TEMPERATURE ON THE GROWTH OF *MACROPHOMINA PHASEOLINA* ISOLATES

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Summary

The charcoal root disease caused by *Macrophomina phaseolina* (Tassi) Goidanich may cause considerable damages in a hot as well as in dry seasons. The effect of temperature was investigated on the growing patterns of 35 *Macrophomina phaseolina* isolates. collected from different districts of Hungary. The fungal cultures in 90 mm Petri dishes held on 10, 15, 20, 25, 30, 35 and 40 °C were measured in four replications. For the isolates the most favourite temperature regime was 25 to 35 °C. At these temperatures mycelium and microsclerotial growing in diameter of all isolate colonies reached 90 mm on the 5th day. Mycelia growth of this pathogen was very low at 10, 15 and 40 °C, and they did not form microsclerotia. The cultures were well grown at 20 °C, their colony size on the 3rd day were 14 times larger than at 10 and 15 °C. Even the extreme temperatures (10 and 40 °C) were not lethal, cultures started to grow when were kept to 25 °C.

USE OF RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) TO DETERMINE VARIATION IN PATHOGENICITY AMONG *EXSEROHILUM TURCICUM* ISOLATES OBTAINED FROM MAIZE AND SORGHUM IN EGYPT

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Maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench) are two of the most important cereal crops in Egypt. Maize and sorghum are subject to infection by some leaf spotting diseases, that reduce the ability of the plant to grow in a normal manner affecting the productivity. One of these diseases is northern corn leaf blight, caused by *Exserohilum turcicum* (Pass.)K.J. Leonard and E.G. Suggs syn. *Helminthosporium turcicum* Pass. (perfect stage is *Steosphaeria turica*). This disease is considered the most important leaf disease in Egypt (El-Shafey, 1978 and Gouda, 1996). Crop losses may exceed 50% if infection becomes severe prior to flowering (Raymundo and Hooker, 1981).

Molecular techniques based on the polymerase chain reaction (PCR) have been used as a tool in genetic mapping, molecular taxonomy, evolutionary studies and diagnosis of several fungal species (Williams *et al*. 1990 and McDonald, 1997). Random amplified polymorphic DNA (RAPD) analysis also may be useful in identifying races and subgroups of plant-pathogenic fungi (Assigbetse *et al*., 1994). A comparative study of genetic diversity in *S. turcica* populations in tropical and temperate climates revealed a higher level of diversity in the tropics (Borchardt *et al*., 1998a). To investigate the pathogen`s population genetic structure in central Europe, Borchardt *et al.* (1998b) sampled 80 isolates of *Setosphearia turcica* from Germany, Switzerland, France, Austria, and Hungary, using 52 random amplified polymorphic DNA(RAPD) markers. Among the 73 isolates from maize, there were 26 different RAPD haplotypes. Haplotype shared by most members was represented by 22 isolates from Germany, Switzerland, and France, indicating high fitness and substantial migration.

The main aim of this investigation is to investigate the variability in the fungal population prevailing in the farmer's fields and realize the relation

between pathogen isolates from different sources of hosts (maize and sorghum) and locations using RAPD technique.

Materials and methods

Molecular marker analysis

DNA isolation:

Eleven *Exserohilum turcicum* isolates (five from maize namely ,Nub.TCL; Sak.5TC; Sam.ITC; Man.TC and Giz.4TC and 6 from sorghum namely, Sak.3TS; Nub.9TG; Qal.TS; Sam.TS; Man.TS & Sed. TS) were devided into four groups according to their pathogenicity reaction to both maize and sorghum.These isolates included two races ,one of them broke down the monogenic resistance gene of maize Pioneer single cross 3080 while the other did not (previous data of the authors) . Method of Murray and Thompson (1980) was used for Fungal DNA isolation.

Random Amplified Polymorphic DNA (RAPD):

Random amplification of DNA sequences was performed with a seven 10-mer primers obtained from Pioneer Company:

A: 5` GGT GCG GGA G 3`
B: 5` GTT TCG CTC C 3`
C: 5` GTA GAC CCG T 3`
D: 5` AAC AGC CCG T 3`
E: 5` AAC GCG CAA C 3`
F: 5` CCC GTC AGC A 3`
G: 5` GGA AGG CTG T 3`

A modification of the RAPD method of Williams *et al*. (1990) was used to perform the amplification.

Analysis of RAPD data:

Digital pictures of RAPD fingerprints were scored manually as “1” for the presence of a band and “2” for the absence of a band, assuming that bands with the same molecular size in different individuals were identical. Each band was treated as a single independent locus with two alleles and unresolved band or missing data were scored as "0". The unweighted pair grouping by mathematical averages (UPGMA) of TFPGA 1.3 (Miller, 1997) was used to construct phenograms (dendrograms) and to estimate genetic distance among isolates using option "Nei (1978) unbiased minimum distance". Cluster analysis was conducted for each primer individually at first and pooled data from all tested primers were analyzed for evidence of sub grouping. Bootstrap analyses (1000 iterations) were conducted on the resulting UPGMA to assess support for any resulting sub groupings.

Results

RAPD analysis of eleven *E.turcicum*-isolates, 5 from maize and 6 from sorghum, was carried out to study the genetic diversity. A total of 66 loci resulted from DNA amplifications of the eleven *E. turcicum* isolates using seven RAPD primers were scored (Figure1). A little more than 71% (47 loci) of the amplified loci were polymorphic where 19 loci (28.8%) were invariant among all isolates in 1:3 ratio. Trees generated from each primer data using UPGMA cluster analysis had very characteristic patterns which are not similar to each other (Figure 2). While, tree constructed from pooled data were more phylogenetically informative (Fig 2H). UPGMA cluster analysis separated the maize isolate; Nub.TCL (group IV) from examined isolates at approximately 43% dissimilarity, and divided the remaining isolates into two clusters, A (group I&II) and B (group III). Although, group III was separated from cluster A at lesser than 30% dissimilarity, it contained two isolates; Man.TC from maize and Sak.3TS from sorghum, with more than 22% dissimilarity. Also, cluster A was divided at about 20% dissimilarity to two groups I and II. Group I gathered three isolates from maize; Sak.5TC, Sam.TC and Giz.4TC, with 15% dissimilarity, while group II contained five isolates, all from sorghum; Qal.TS, Sed.TS, Nub.9TG, Sam.TS, and Man.TS, with similarity of about 85% (Figure2H).

Figure1. Random amplified polymorphic DNA(RAPD) profiles of 11 *E. turcicum* isolates obtained by using primers: A, B, C, D, E, F and G, respectively. Lanes contain: M, DNA 1KB ladder (Biolab) for A, B, C, D, E and F; and φX174 DNA/HaeIII (Promega) for G; maize isolates 1, Nub.TCL; 2, Sak.5TC; 3, Sam.1TC; 4, Man.TC & 5, Giz.4TC; and sorghum isolates 6, Sak.3TS; 7, Nub.9TG; 8, Qal.TS; 9, Sam.TS; 10, Man.TS & 11, Sed.TS.

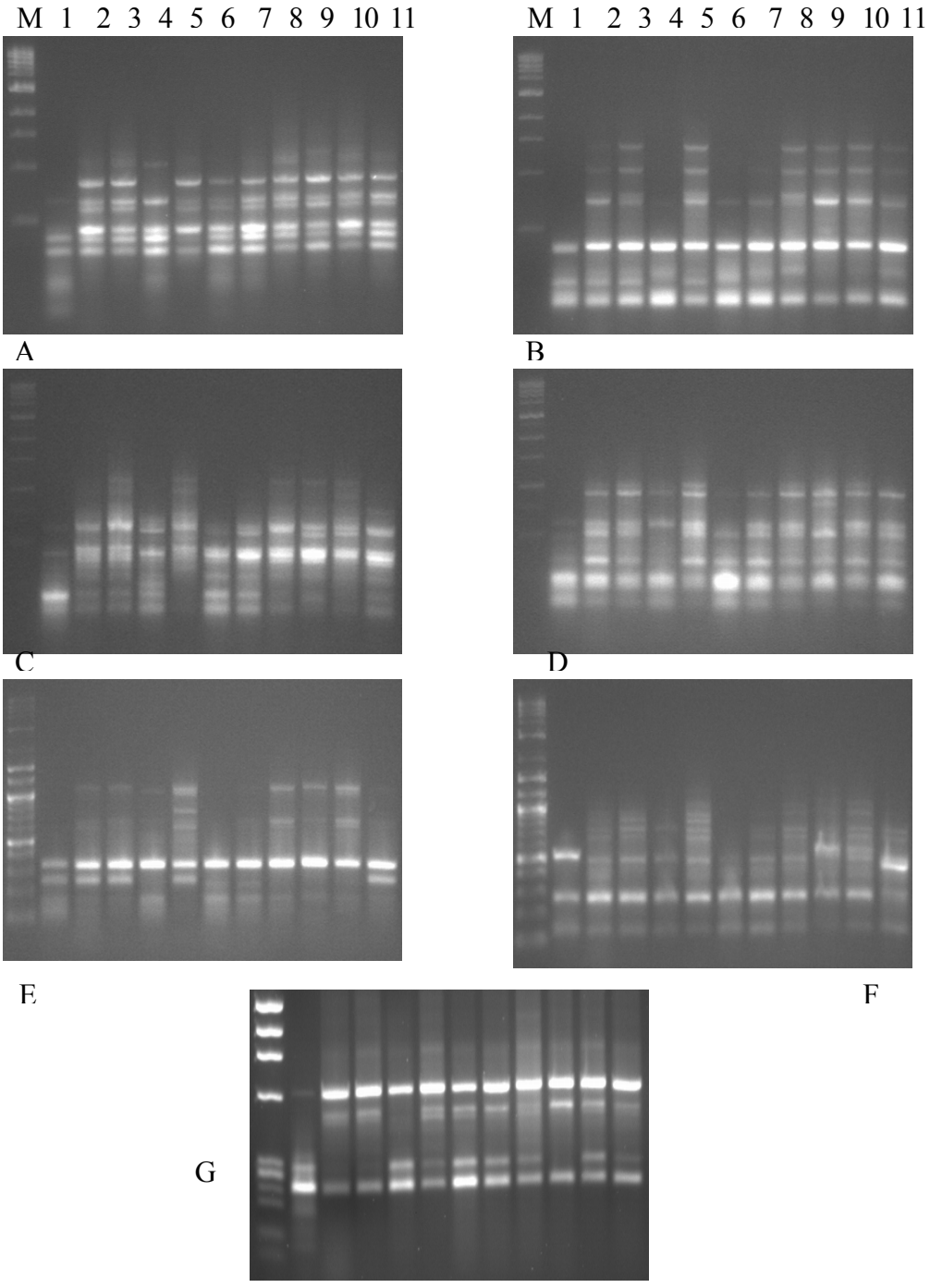
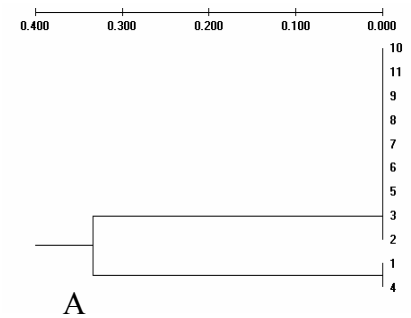
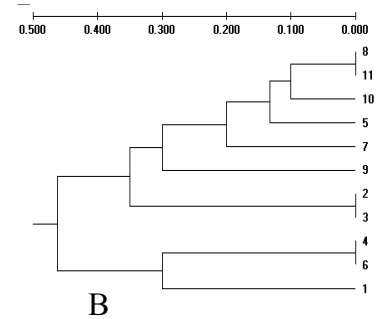


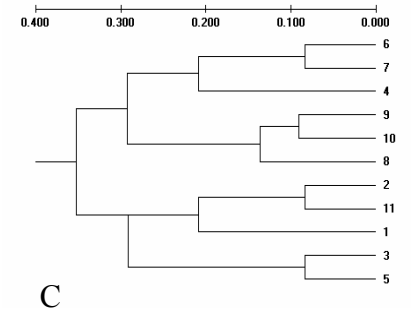
Figure2 Dendrogram generated from cluster analysis of eleven isolates of *E. turcicum,* isolated from maize (1-5) and sorghum (6-11), using UPGMA, unbiased minimum distance (Nei 1978). Trees A, B, C, D, E, F, and G are generated from individual RAPD primer data, while H represents all previous data combined.



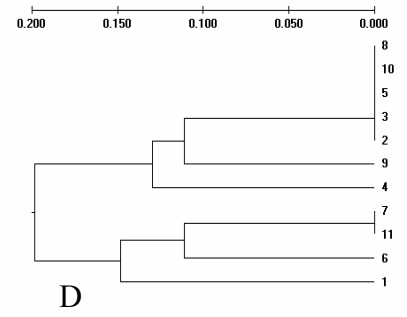
A



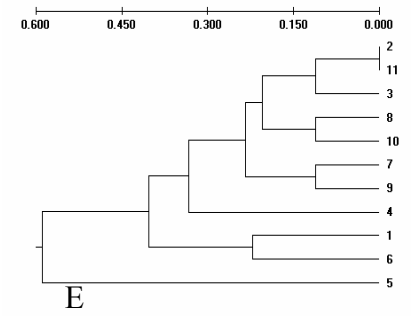
B



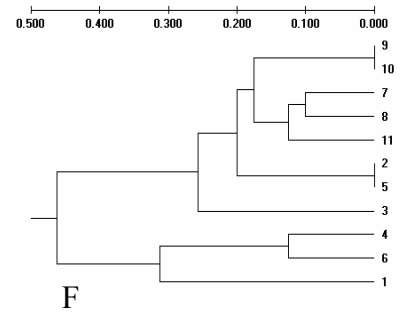
C



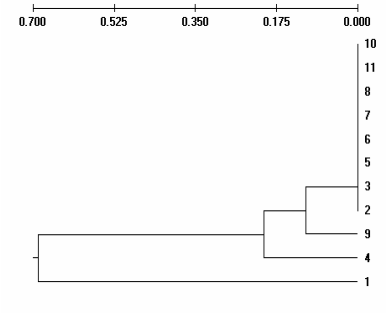
D



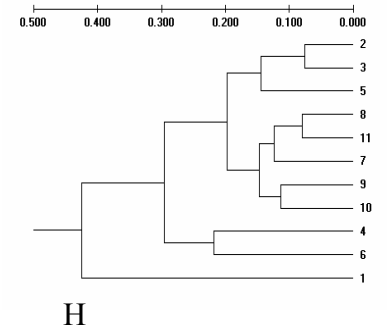
E



F



G



H

In RAPD analysis of *E. turcicum,* the seven 10 mere primers used produced 66 loci of which 47 (71%) were informative (Polymorphic). Therefore, the large number of useful RAPD markers permits a more robust statistical analysis and, presumably represents a greater coverage and dispersal of markers across the genome. RAPD polymorphism analysis of individual primer data set was shown to has poor correlation with most isolate characteristics (i.e. geographic distribution, mating type …etc.); although the sample size was small for such analysis. However, based on RAPD polymorphic pooled data, four distinct groups were identified within the eleven *E. turcicum* tested isolates. Group I gathered three maize isolates; Sak.5TC, Sam.1TC and Giz.4TC (Figure 2), all of which were capable of causing more than 40% disease severity on the TWC 310 maize hybrid. On the other hand, the sorghum isolates; Qal.TS, Nub.9TG and Sam.TS that were incapable to attack TWC 310 with more than 4.2% disease severity were separated in group II (Figure 2H). Group III gathered 2 isolates which have different degrees of aggressiveness on maize and sorghum, 1 from maize which could infect maize weakly but was unable to infect sorghum, and the other from sorghum, which severely attacked both hosts. Group IV, however, included just one isolate from maize. This isolate has the efficiency to break down the monogenic resistance SC30809(obtained data by the authors, under publication). Accordingly, this work separated clearly a new race (group IV) from the other isolate groups.

These results are consistent with some extent to those obtained and discussed by Ferguson and Carson (2004) . Similarities and differences in banding patterns obtained by RAPD could be a useful molecular tool in evolutionary studies of the new race (group IV) (Fig 2H).

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USE OF RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) TO DETERMINE VARIATION IN PATHOGENICITY AMONG *EXSEROHILUM TURCICUM* ISOLATES OBTAINED FROM MAIZE AND SORGHUM IN EGYPT

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Summary

Northern corn leaf blight caused by *Exserohilum turcicum* is considered the most important foliar disease of maize and sorghum in Egypt. Eleven isolates of *E.turcicum* from maize and sorghum which were previously evaluated for their pathogenicity against maize and sorghum by the authors, were used for RAPD analysis. Tree constructed from pooled data of seven RAPD primers using UPPGMA cluster analysis divided the examined isolates of *E. turcicum* into 4 groups. The fourth group contained a new race which overcome the monogenic resistance gene in maize single cross 3080.

METHOD FOR BIOLOGICAL CONTROL ON CHESTNUT TREES IN SLOVAKIA

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Chestnut blight disease, caused by the fungus *Cryphonectria parasitica* (Murrill) Barr can be controlled by means of a biological method based on the use of hypovirulent strains of the pathogen. This method is used in many regions of Europe to protect *Castanea sativa* Mill. against the pathogen. Hypovirulence comprises all abnormal conditions which reduce the fitness of the pathogen, including infection by hypoviruses (Elliston, 1982).
Cryphonectria parasitica, the chestnut blight fungus occurs in the nature in two forms: virulent and hypovirulent. Hypovirulent strains have different physiological and morphological properties (Grente 1965). Hypovirulent isolates are discoloured (white mycelia in cultures), practically without reproduction organs (lower sporulation), and with significantly less virulence to *Castanea sativa,* compared to the virulent isolates. The trees respond to the hypovirulent strains by promoting callus formation and healing of the cankers and development of the callus. Abnormal cankers from which the hypovirulent strains of the fungus are isolated consist of exposed sapwood bordered by vigorous callus, with superficial infections radiating from the margins of the openings (Elliston, 1985). Cytoplasmic hypovirulent strains consistently containing dsRNA (Anagnostakis and Day 1979). The final effect of the hypovirulent agent (dsRNA hypovirus) is to reduce mortality of infected trees. The chestnut blight has recently been controlled by means of a biological method based on the use of these hypovirulent strains of *C. parasitica*.

Materials and Methods

Bark samples (4–5 cm) were cut from chestnut blight cankers. The samples were immersed in 0.15% NaClO solution for 20 minutes for surface disinfection, and subsequently washed in distilled water. Small pieces of bark chips (cca 0.5 x 0.5 cm) were placed on 3% malt agar. The isolates were incubated at 25–27 °C in the dark.
According to Grente (1981), Grente and Berthelay-Sauret (1969a, b), virulent and hypovirulent isolates of *C. parasitica* are morphologically different in culture and distinguishable to the naked eye. Mycelium of the

virulent isolates is white, later turning to yellow or orange-yellow. About 96–140 hours after subculturing, globose red-orange pycnidia are seen in the culture. They are produced abundantly. In the hypovirulent isolates, the mycelium remains white and the production of pycnidia is very low.
Conversion of virulent isolates of *C. parasitica* with hypovirulent strains was done on malt agar. One pair of media cubes with mycelium (one virulent and one hypovirulent) was placed in contact on the medium in a Petri plate, about 5 mm from the edge of the plate. The conversion is successful when the white mycelium is divided in virulent mycelium close to the interface with hypovirulent strain. The test was replicated 10 times. The aim of this test was to transfer dsRNA hypovirus from the hypovirulent strains into the virulent isolates and, in such a way, to obtain domestic hypovirulent isolates that can be used in biological control of chestnut blight.

Results and Discussion

The samples were taken from different parts of infected trees (from attacked bark, from mycelium and also from pycnidia). All isolates had at first white-coloured mycelium. Later it turned to light yellow and orange-yellow, some weeks later, to red-orange. After 5 days, pycnidia were formed in cultures. They were produced abundantly. Based on phenotype of this culture, all isolates were considered to be virulent. No hypovirulent isolates has been detected in Slovakia so far.
Slovak virulent isolates were transformed into hypovirulent forms with French hypovirulent isolates (Table 1) and with Hungarian hypovirulent isolates, (described by Radócz, 2001) (Table 2) and were used for biological control of chestnut blight in Slovakia.
In 2004 53 isolates with morphological properties of hypovirulent strains were obtained from naturally healing cankers. Present of dsRNA using molecular method was confirmed in eight of them. It means that hypovirus was spreading to normal cankers in the natural way, and an important success in chestnut protection against chestnut blight has been achieved.

Table 1: Results of conversion of Slovakian virulent isolates with French hypovirulent strains within the 1990-1993.

	French hypovirulent strains									
Locality	2022	2029	2079	2099	2100	2103	2104	2106	2113	2073
Radošina	-	-	-	-	-	-	-	+	-	-
Bratislava	-	-	-	-	+	-	+	-	-	-
Myslenice	-	-	-	-	-	-	-	+	-	-
Pezinok	-	-	-	-	+	+	+	-	-	-

+ successful conversion of virulent isolates
- unsuccessful conversion of virulent isolates

Table 2: Results of conversion of Slovakian virulent isolates with Hungarian hypovirulent strains within the 2004-2006.

Locality	Hungarian hypovirulent strains												
	A3xBF	C	C-2	R-6	R-5	R5x2	FS8 W31	FS4 146	R11	V4	IHB 2	R-2	B1xBF
Pernek	-	+	+	-	+	-	-	-	-	+	-	-	-
Petrovce	+	-	+	+	+	+	-	-	-	+	+	+	-
Hlohovec	-	-	-	-	-	-	+	+	-	-	-	-	-
Stredné Plachtince	-	-	-	+	+	-	-	-	-	-	-	-	-
Modra	-	-	-	-	-	+	-	-	-	-	+	-	-
Príbelce	-	-	-	-	-	+	-	-	-	-	-	+	-
Senec	-	-	-	-	-	-	-	-	-	-	-	-	-
Arborétum Mlyňany	-	+	-	-	-	-	-	-	-	-	-	-	-
Horné Plachtince	-	-	-	-	-	-	-	-	+		+	-	-
Dolné Štitáre	-	-	-	+	-	+	-	-	+	-	+	+	-
Horné Lefantovce	-	-	-	+	-	+	-	-	-	-	-	-	-
Párovské Háje	-	-	-	-	-	-	-	+	-	-	-	-	-
Veľké Lovce	-	+	+	+	-	-	-	-	-	+	+	+	-
Krnča	-	+	-	-	-	-	-	-	-	-	-	-	-
Sv. Jur	-	-	-	+	-	+	-	-	-	-	-	-	+
Modrý Kameň	-	-	-	+	+	-	-	-	-	-	+	-	-
Bratislava	-	+	-	-	+	-	-	-	-	-	-	-	-
Horňany	-	-	-	-	-	-	-	-	-	+	-	-	-
Stráne	-	-	-	-	-	-	-	-	-	-	+	-	-
Svodín	-	-	-	-	-	-	-	-	-	+	-	-	-

+ successful conversion of virulent isolates

- unsuccessful conversion of virulent isolates

Acknowlegment
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Summary

Cryphonectria parasitica, the chestnut blight fungus occurs in the nature in two forms: virulent and hypovirulent. The hypovirulent strains have different physiological and morphological properties. Hypovirulent strains of the pathogen can be used in biological control against the diseases. All strains of the fungus isolated from different parts of infected trees were considered to be virulent based on phenotype of culture. No natural hypovirulent isolates has been detected so far in Slovakia. Slovakian virulent isolates were transformed into hypovirulent forms with French and Hungarian hypovirulent isolates, and were used for biological control of chestnut blight in Slovakia. In 2004 isolates with morphological properties of hypovirulent strains were obtained from naturally healing cankers.

MICROSCOPIC FUNGI ASSOCIATED WITH HORSE-CHESTNUT LEAVES

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Leaf surfaces are an important habitat for the growth of microorganisms. The leaf surface mycofloras of various plants have been extensively reviewed (Preece, Dickinson 1971, Dickinson, Preece 1976). Many studies dealing with the distribution patterns of fungal communities have been performed in recent decades: host specificity in fungal populations have been widely surveyed (Boddy, Griffith 1989, Petrini, Fisher 1990, Kowalski 1991, Collado et al. 2000). Comparative studies of fungal populations in healthy and symptomatic tissues have been performed (Hata, Futai 1995, Radócz, 1999, Ragazzi et al., 2003). The presence of fungi in healthy plant tissues has been commonly observed (Carroll 1988). The main aim of the present study was to determine the composition of the fungal assemblages of healthy or symptomatic horse-chestnut leaves.

Materials and Methods

Living leaves of European horse-chestnut (*Aesculus hippocastanum*) were collected during the vegetation period to isolate microscopic fungi. After surface sterilization the leaves were cut into several fragments that were then placed in Petri-dishes containing 2% Malt extract agar (MEA). The plates were incubated at 22±2°C. After incubation for 15 or more days, individual fungal colonies were picked from the edge with a sterile fine tipped needle and transferred onto 2% MEA plates. The fungi were identified based on cultural and morphological characteristics. The isolation frequency (IF) of a single fungal taxon was calculated by the following formula: $IF = N_i / N_t \times 100$, where N_i and N_t are the number of segments from which the fungus was isolated and the total number of segments cultured, respectively.

Results and Discussion

A total of 12 species were isolated from horse-chestnut leaf samples (Table 1). These fungi included members of two genera of Ascomycetes and ten genera of Deuteromycetes. In leaves they were (in order of decreasing frequency): *Phyllosticta sphaeropsoidea*, *Phomopsis carposchiza*, *Colletotrichum gloeosporioides*, *Asteromella aesculicola*, *Trichothecium*

roseum, Alternaria alternata, *Cladosporium cladosporioides*, *Epicoccum nigrum*, *Phoma* sp., *Chaetomium* sp., *Diaporthe padi* and *Trichoderma polysporum*.

Table 1: Isolation frequencies (IF)[1] of fungal species in horse-chestnut leaf samples

Fungal species	IF	Fungal species	IF
Alternaria alternata	11.0	*Epicoccum nigrum*	6.0
Asteromella aesculicola	16.5	*Phoma* sp.	4.0
Cladosporium cladosporioides	6.5	*Phomopsis carposchiza*	18.5
Colletotrichum gloeosporioides	17.0	*Phyllosticta sphaeropsoidea*	47.5
Chaetomium sp.	2.0	*Trichoderma polysporum*	1.0
Diaporthe padi	1.5	*Trichothecium roseum*	13.5

[1]The isolation frequencies for each species are the percentages with respect to total number of fragments cultured.

A majority of the fungal species were recovered from necrotic tissues. Furthermore, *Alternaria alternata*, *Cladosporium cladosporioides*, *Epicoccum nigrum*, *Phomopsis carposchiza* and *Trichothecium roseum* were also recovered from healthy ones. *Phyllosticta sphaeropsoidea*, well-known horse-chestnut pathogen and with a potential for causing severe damage to horse-chestnut leaves (Zimmermannová, 2001), was very frequently isolated in most of the sampling sites and was recovered from necrotic plant tissues.

Changes in diversity and equitability with increasing leaf age could be due to various factors. Weathering of leaf cuticle, wounds, insect attack and increased exposure time have been suggested to account for the increase in diversity as leaves age (Carroll 1979, Forster 1977). The host specificity among endophytes is expressed at the family level. However, some endophytic genera such as *Phyllosticta*, *Phomopsis* and *Colletotrichum* occur in a wide variety of distantly related host species (Toofanee, Dulymamode 2002).

The use of the two different approaches (sterilized and unsterilized fragments) may detect the living conditions of the different species: whether it lives as an epiphyte or as an endophyte; whether the fungus inhabits only the surface of the tissue or grows within the tissue. According to Santamaría, Diez (2005) the isolation frequencies from necrotic and healthy plant tissues were significantly different between collection sites and isolation methods. Several workers have recognized that leaf age exerts great influence on the composition of leaf surface microorganisms (Dickinson 1967, Sharma, Mukerji 1973).

Our results show that horse-chestnut leaves have a diverse fungal flora. Further investigations on the fungal species associated with horse-chestnut leaves during different seasons and increased sampling effort could yield more fungal taxa.

Acknowledgements
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MICROSCOPIC FUNGI ASSOCIATED WITH HORSE-CHESTNUT LEAVES

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Summary

In this study, leaves of *Aesculus hippocastanum* were screened for composition of fungal communities. The most prevalent fungi associated with healthy and symptomatic horse-chestnut leaves were (in order of decreasing frequency): *Phyllosticta sphaeropsoidea*, *Phomopsis carposchiza*, *Colletotrichum gloeosporioides*, *Asteromella aesculicola*, *Trichothecium roseum, Alternaria alternata*, *Cladosporium cladosporioides*, *Epicoccum nigrum*, *Phoma* sp., *Chaetomium* sp., *Diaporthe padi* and *Trichoderma polysporum*. *Phyllosticta sphaerospoidea* was found to be the dominant species. *Phomopsis carposchiza* and *Colletotrichum gloeosporioides* were also present in great abundance. Our results suggest that the mycoflora of horse-chestnut leaves is heterogeneous.

THE ANTHRACNOSIS DISEASE OF THE VARIABLE LUPIN (*LUPINUS MUTABILIS SWEET*)

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In connection with the lupin (*Lupinus* L.) species *(Lupinus albus, L. luteus, L. angustifolius, L. mutabilis)* which are grown in Hungary or used for plant improvement, we have experienced a previously unknown disease since 2004, namely the anthracnosis. The extent of damage caused is different for each species, the biggest loss was experienced with regard to the white and yellow flowered sweet lupins that are also grown on an industrial basis. To provide a precise definition about the extent of the damage, further years of observation are essential.

The variable lupin – as far as we know – is only infected by the *C. gloeosporioides*. The tight leaf or blue flowered lupin species are – as far as we are concerned – only infected by the *Colletotrichum gloeosporioides*.

To decrease the impact of this agent we find – on the basis of our fungicid-sensitivity surveys – captan, mankoceb, copper(I)oxid as the most suitable as well as the combinations of these with benomyl or methyl-tyophanate. Our open-air small-parcelled experiments are to be settled according to this.

Effective protection is impeded by the fact that this pesticide also affects the inside part of the seed in the case of the lupin species examined. Seed pelleting is only able to provide a partial protection, which means that stock treatments are assumed to have an important role in the future in the plant protection of this species.

In relation to heat demand, the causative fungus agent belongs to the warm demanding species. If the humidity and the temperature is high enough – considered as an ecological condition – that may nourish the fungicid infection of this lupin species, which, migh as well be ”epidemic”!

OCCURRENCE OF *PUCCINIA HORDEI* ON WINTER BARLEY IN HUNGARY IN 2006

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Cultivated barley ranks as fourth in world cereal production, and is the third important agricultural species in Hungary.
The aim of barley breeding at Kompolt is to develop high yielding cultivars resistant to diseases for malt and feed. One of diseases of barley, the leaf rust, caused by *Puccinia hordei* G. Otth., widespread around the world, but occurs not every year in Hungary.

We surveyed the occurrence of *Puccinia hordei* in Hungary in 2006, and tested 28 winter barley cultivars for resistance to leaf rust.

We noticed, leaf rust was an important disease on winter barley in West-Hungary at Szombathely, and occurred in traces in North-Hungary at Kompolt.

The level and type of resistance against leaf rust were determined in cultivars. Disease susceptibilities of winter barley cultivars ranged from very susceptible to resistant. Among the cultivars, few showed high resistance (e.g. Botond, Lambic) and some were very susceptible (e.g. KH Agria, KH Turul, Petra). The leaf rust was in traces on few cultivars.

This study presents the first results about resistance to leaf rust of barley cultivars in Hungary. These data help the Hungarian barley breeders in rust resistance breeding program.

*PHOMA*S – CAN THESE FUNGI BE USED AS BIOCONTROL AGENTS AND SOURCES OF SECONDARY METABOLITES? (A REVIEW)

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Introduction

Phoma is a genus containing more than 2000 described species. This genus traditionally refers to simple stem inhabiting pycnidial fungus with small hyaline, unicellular pycnidiospores (Sutton, 1980). *Phoma*s cause various serious diseases to plants as well as to humans (Rai 1989, 2000). Besides these harmful aspects, certain *Phoma* species also contains antibiotic potential and economically useful secondary metabolites.
As widespread plant pathogens around the world occur on a broad range of plant species, e.g. *P. glomerata* has been reported on grape, potato, wheat, pear, mango, rice and many other crops. Some important example include *Phoma lingam* that causes the very serious blackleg disease in canola. It is also found on other cruciferous crops such as cabbages, cauliflower and summer rape. *P. medicaginis* var. *medicaginis* is the causal agent of spring black stem of alfalfa. *P. pinodella* causes foot rot in peas and black stem in clovers. All of these pathogens can overwinter in crop debris and are often seedborne. Development of disease symptoms caused by these *Phoma* spp. will often be enhanced in wet and cool conditions. Within this genus large pathogenic variations are shown which often complicates the control of *Phoma*, especially in legumes.

The present review paper is aimed to discuss the role played by *Phoma* species as biocontrol agents and their potential for the production of secondary metabolites.

*Phoma*s as biocontrol agents in weed control

Weeds are serious problems not only for agricultural and forestry fields, but also responsible for several major problems to human and animal health around the world including India. Synthetic chemical herbicides have been the mainstay for weed control practices since the end of World War II and no doubt are responsible for much of the unparalleled increased crop productivity that has occurred during this period. The high costs involved in developing and registering chemical herbicides and recent trends in environmental awareness have prompted researchers to investigate alternative systems of weed control. Ideally, such a system would control target weeds at or near the same levels as that achieved with chemical herbicides while not poisoning a threat to either the environment or non-target organisms at the same time (Pandey et al., 2001).
The science and technology of weed control by using plant pathogens more especially fungal pathogens as an effective alternatives to chemical herbicides have gained significant attention and momentum in 1970. Century old concept in weed control and plant disease epidemiology were successfully put to text and few economically important weeds were controlled by fungal pathogens used under classical and mycoherbicidal strategies. With the advancement in the knowledge biorationals and integrated management strategies have also came into foray. Biological, technological and economical perspectives of various strategies have been extensively reviewed in several publications (Auld, 1990; Charudattan, 1991, 1996; Abbas and Duke, 1995, 1997; Boyette and Abbas, 1995; Hasija et al., 1994; Hoagland, 1990; 1999, 2001; Pandey 1999, 2000; Pandey et al., 1996a,b, 1997, 2001; Saxena and Pandey 2000; Saxena et al., 2001).

As a matter of fact, *Phoma* spp. could be the novel agents for many weed problems. They have both mycoherbicidal and biorational properties. Looking to the number of known species, species associated with weeds are very less. This might be because of ignorance of weed pathogens. Mycologists as well as plant pathologists in the past have given attention to economically important plant diseases. Therefore, urgent attention towards herbicidal potential of *Phoma*s is needed.

It is surprising that despite of excellent phytopathogenic potential shown by various species or varieties of the genus, their mycoherbicidal potential has been ignored significantly. There are only few scanty attempts have been made to evaluate them as mycoherbicides. Heiny (1990, 1994) isolated a highly host specific strain of *Phoma proboscis* from diseased parts of field bindweed (*Convolvulus arvensis*). Heiny and Templeton (1991) have

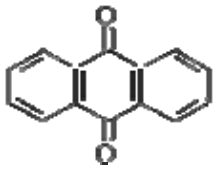
reported very high mycoherbicidal effect when the agent applied to the seedling of the weed and atmospheric temperature ranged from 16-28^{o}C and more than 9 hrs dew period. Heiny (1994) has extensively evaluated the compatibility of synthetic herbicides for integration with the mycoherbicidal agent.

Rajak et al. (1990) isolated a strain of *P. herbarum* from diseased leaves of *Parthenium hysterophorus* L. collected from Central India. The fungus caused more than 90% inhibition in seed germination, seedling mortality and leaf damage followed by reduction in height of *Parthenium* (Pandey et al., 1991). Pandey and Pandey (2000) recovered three strains of *P. herbarum* (LC#32,37,39) from diseased leaves and stem of *Lantana camara.* All the three strains incite severe infection in the weed, especially at seedlings stage (Pandey, 2000). Pandey (2002) also isolated a strain of *P. herbarum* FGCC#70 from disease seedlings of an invasive weed, *Hyptis suaveolens* (L.) Poit. He recorded very high mycoherbicidal potential when seedlings were treated. Several other species viz. *Phoma campanulata* on *Cassia fistula* (Rajak and Rai, 1982), *P. exigua* on *Sesamum indicum* (Singh and Agarwal 1973), *P. eupyrena* on *Achyrenthus aspera* (Khanna and Chandra, 1977), *P. glomerata* on *Crotalaria juncea* (Pathak and Chauhan, 1976), and *Parthenium hysterophrous* (Padmbai, 1976*), P. lantanae* on *Lantana camara* (Singh and Agarwal, 1974), *P. palmarum* on *Calotropis procera* (Khanna and Chandra, 1977; Kamal and Singh, 1979), *P. tridocis* on *Tridex procumbens* (Wehmeyer, 1964), *P. herbarum* var. *ipomoeae* (Kamal and Singh, 1979), and *P. euphorbiae* on *Euphorbia hirta* (Rangaswami et al., 1970) have been reported for various parts of India.

Production of anthraquinone pigments by *Phoma* species

There are many fungi which produce anthraquinones (Figure 1A) as secondary metabolites. Fungal anthraquinones as polyketide-derived secondary metabolites occur widely in many genera of fungi. Compared with the commercially available hydroxyanthraquinones most possess an additional methyl substitution in position three, e.g. emodin and this allows a study of the effect of such a group on the dyeing properties of dyestuffs derived from them. A fungal anthraquinone was cynodontin (Figure 1B) produced in sufficient purity to allow it to be transformed using a simple chemical step to a dye product and this was compared with a commercially available close analogue (Hobson et al., 1997).

A/ B/



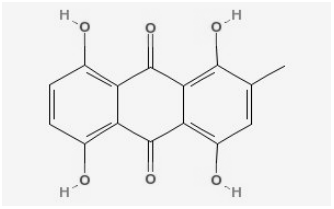


Figure 1. A/ Chemical structure of anthraquinone (anthracene-9,10-dione) and B/ cynodontin (1,4,5,8-tetra hydroxy-3-methylanthraquinone)

The chemical synthesis of anthraquinones require the use of strong acids at high temperature and heavy metal catalysts, as a consequence of which environmentally hazardous effluents and byproducts are produced. With increasing awareness of the environment degradation by industry the disposal of industrial effluent is becoming more costly and strictly regulated. A return to dyes extracted from molluscs, insects, fungi or plants grown in their environment is not the intention.

Phoma exigua Desm. varieties produces pigments. Bick and Rhee (1966) have reported that *P. exigua* var. *foveata (=P. foveata)* contains many anthraquinone pigments, such as pachybasin (Figure 2), chrysophanol (Figure 3), emodin (Figure 4) and phomarin. In acid condition this complex of pigments becomes yellow, and in alkaline conditions red. This character is based on ammonia test described by Logan and Khan (1969). On malt-agar, *P. exigua* var. *foveata* gives pinkish colour after exposure to ammonia. This is due to reaction of diffusible anthraquinones and their reaction with ammonia. In old cultures, anthraquinone pigments crystallize out as yellow-green crystals. Tichelaar (1974), found that the fungicide thiophanate-methyl accelerates and increases the crystallization process of the pigments. Both *P. exigua* var. *exigua* and *P. exigua* var. *inoxydabilis* produce cytochalasin B, which are also known as “phomine” (Bousquet and Barbier, 1972; Scott et al., 1975).

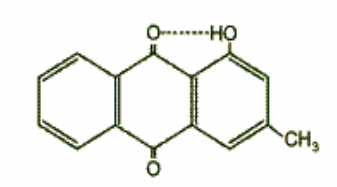


Figure 2. Pachybasin (1-hydroxy-3-methylanthraquinone)

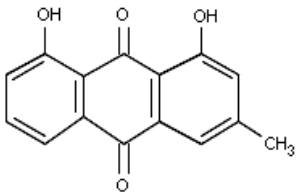


Figure 3. Chrysophanol (1,8-dihydroxy-3-methylanthraquinone)

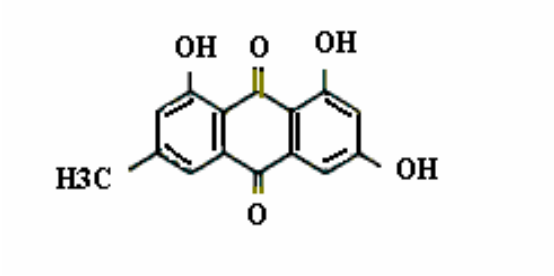


Figure 4. Emodin (1,6,8-trihydroxy-3-methyl-anthracene-9,10-dione)

Some isolates of *P. exigua* var. *foveata* (=*P. foveata*) produce antibiotic substance ('E' metabolite) similar to isolates of the ubiquitous *P. exigua* var. *exigua* (Boerema and Höweler, 1967). It is a colourless substance and can easily be demonstrated in cultures by sodium hydroxide test. On application of a drop of sodium hydroxide at the margin of colonies on malt agar oxidation takes place and pigment alpha (∝) converts into pigment beta (β). Pigment alpha (∝) is red-purple at pH <10.5 and blue-green at pH >12.5. Pigment beta (β) is yellow at pH <3.5 and red at pH >5.5.

Recently, three known anthraquinones have been isolated and identified by Borges and Pupo (2006), 1,7-dihydroxy-3-methyl-9,10-anthraquinone (Figure 5), 1,6-dihydroxy-3-methyl-9,10-anthraquinone (Figure 6) and 1-hydroxy-3-methyl-9,10-anthraquinone (Figure7), one new anthraquinone (1,7-dihydroxy-3-hydroxymethyl-9,10-anthraquinone), and two new hexahydroanthra-quinone derivatives, dendryols E and F (Figure 8), were isolated from the culture of the endophytic fungus *Phoma sorghina*, found in association with *Tithonia diversifolia* (Asteraceae). Their structures were identified on the basis of spectroscopic data, mainly 1D and 2D NMR.

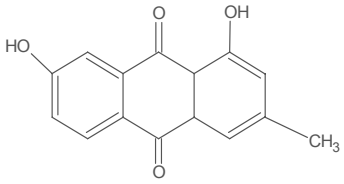


Figure 5. 1,7-Dihydroxy-3-methyl-methyl-9,10-anthraquinone

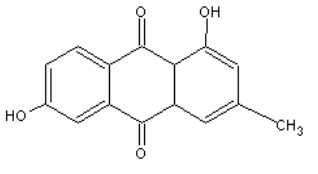


Figure 6. 1,6-Dihydroxy-3-9,10-anthraquinone

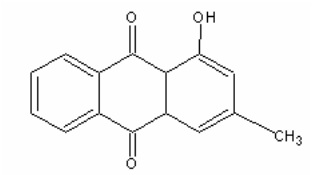


Figure 7. 1-Hydroxy-3-methyl-9,10-anthraquinone

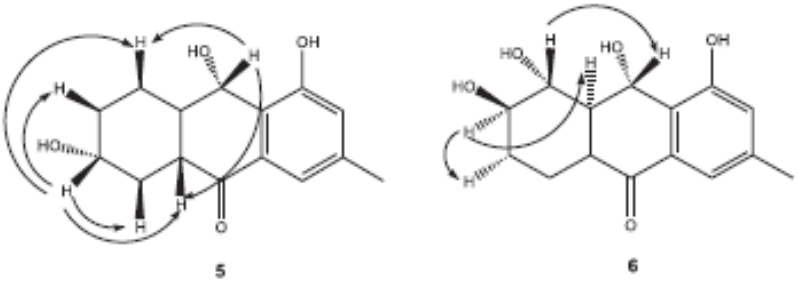


Figure 8. Antraquinone derivates produced by the endophytic fungus *P. sorghina*, the main correlation observed in the NOE different experiments for dendryol E and F (5-6) (after Borges and Pupo, 2006).

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COMPARATIVE SEED PATHOLOGICAL INVESTIGATIONS ON CULTIVATED GRASS SPECIES

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The grass area of Austria amounts to 2 million hectare, of which 47 % is cultivated included the fodder-plant production. The other part is extensive grass (alpine pastures, mountainous areas). In average a yearly 86.000 ha new grass area will be made by sowing, oversowing and intercropping. For these purposes 5.545 tons of grass seed has been used between 2002 and 2004. For a high level production it is necessary to have healthy, pathogen free seed with excellent quality. However we have not enough information on seed contaminating fungal pathogens, and about their effect on the germination. The aim of our work was to investigate the seed pathology of common bent-grass (*Agrostis capillaris* L), yellow oat-grass (*Trisetum flavescens* L.) and the crested dog's-tail (*Cynosorus cristatus* L.) with special respect toward the connection of fungal contamination and germination of the seeds. These three grass species constitute just a small part of the grass seed production, still they play an important role in the plant community (phytocoenosis) of the higher mountain areas.

Simay (1989, 1990, 1991) investigated seed pathology of a number of plant species, but among them only oat and maize belong to the *Poaceae* family (Simay 1992a, 1992b). He did not investigate grass seed pathology. Walcz and Horváth (1976) made seed pathology tests of three grass species (smooth brome, red fescue and tall fescue) in some years. They used different test methods, and found, that the occurrence of *Alternaria alternata* is the most important pathogen on grass seeds. Tagenko (1974) found also *Alternaria alternata* on seeds of smooth brome. Beside *A. alternata* and *A. tenuissima* Gannibal (2004) identified *A. infectoria* on seeds of different grass species. He reported also data on occurrence of *Ulocladium* and *Embellisia* genus, genera near to that of *Alternaria*. Varga et al. (2004) investigated seeds of 30 cultivars from 11 grass species. They pointed out dominance of *Alternaria* genus as seed pathogens. Within the genus *Alternaria alternata* and *A. tenuissima* were identified and the occurrence of *Ulocladium* genus on seeds of perennial ryegrass also was reported. Similarly, genera of *Embellisia* and *Septonema* were identified from seeds of perennial ryegrass (Varga and Fischl 2005). Makela (1972) found in his investigations a number of grass species, where the seed of

common bent-grass was partly contaminated. Papp et al. (1986) found fungal species of *Fusarium graminearum, Bipolaris sorokiniana, Septoria graminum, Colletotrichum graminicola* and *Mastigosporium album* to have an importance on yellow oat-grass seeds. Radulescu and Negru (1971) also mentioned these pathogenes from yellow oat grass seeds. They referred to the presence of *Fusarium nivale, F. insidiosum*, *Drechslera erythrospila* and beside them *Curvularia lunata* on seeds of common bent, and verified also the presence and distribution of more *Tilletia* and *Ustilago* species. The above mentioned authors did not give data on fungal pathogenes distributed and spread by crested dog's-tail seeds.

Materials and Methods

The investigations were made at the area of HBLFA Raumberg-Gumpenstein Research Institute, in Austria. The tested seed lots of common bent-grass (*Agrostis capillaris* L), yellow oat-grass (*Trisetum flavescens* L.) and the crested dog's-tail (*Cynosorus cristatus* L.) were collected from the 2000, 2001, 2002 year's harvests. The seed samples were stored in paper bags in refrigerated room at 4-6 °C. The samples were not surface sterilized and tested with two different methods considered the prescriptions of the ISTA. Four times 400 seeds were taken onto filter paper in a germination cabinet and also 4 x 400 seeds of each species were tested on a Jacobsen table. Filter papers were moistened with a 0,3 % KNO_3 solution, and in the case of yellow oat grass a cold treatment for 7 days at 4-6 °C were made to overcome their dormancy. During the germination in the cabinet 12 hs light and 12 hs darkness was given and a temperature of 20 °C. During the germination at the Jakobsen table also 12 hs illumination was given, and 30 °C water temperature for the crested dog's-tail and yellow oat grass seeds, and 20 °C in case of common bent-grass. The mycological observations were made twice at the same times of the germination estimations. The observations took place in case of the common bent grass on the 7^{th} and 28^{th} day, in the case of yellow oat grass on the 7^{th} and 21^{st} day, while in the case of crested dog's tail on the 10^{th} and 21^{st} day. Each seed were viewed using a stereomicroscope, and those showing symptoms of fungal contamination, also by a light microscope at 780 x magnification. For the determination of the fungal genus and species the morphology, color, and size of fungal body parts were taken into consideration according to the descriptions of Ellis (1971), Booth (1971), Chidambaram et al. (1973), Sutton (1980) and Sivanesan (1987). Some microphotos were also taken on the different fungal pathogens.

Results and Conclusions

We have found that germination rate of the tested seed samples were excellent, despite the fact that they originated from different harvesting years. The tested grass species showed their germination potential already at the first observation date, and at the second observation time there was just a minimal difference as compared to the first observation for all the three grass species. We have found, that using the Jakobsen table the germination rates of the three species were 2-6 % higher than at the filter paper method. The fungal contamination rate was found to be higher at the Jakobsen table method, as compared to the other one (Figure 1 and 2). We had to notice however, that only contamination rates at the first observation can be considered as real, since in most cases, especially using the Jakobsen table, for the time of the second observation a later infection of the test material by the saprotrophic *Stachybotrys atra* caused the higher data. The fungal contamination rate in case of crested dog's tail was higher, and in case of yellow oat grass and common bent grass was lower. We identified from the seed surfaces 21 fungal genera and their species. The species of the saprophytes genera, *Alternaria alternata, A. tenuissima, Cladosporium cladosporioides, C. herbarum, Stachybotrys atra, Epicoccum nigrum* proved to be the most dominant. From the plant pathogenic genera the *Bipolaris, Drechslera*, and *Fusarium* caused considerable infection rate (Table 1. and 2). In many cases more pathogenes could be identified from a single seed surface. The *Bipolaris sorokinina* were identified from the seeds of common bent grass and crested dog's tail. *Drechslera* species could be identified in case of crested dog's tail and yellow oat grass seeds. Within the *Fusarium* genus the *F. avenaceum* and *F. semitectum* were identified, the infected seeds did not germinate or their sprouts became brown and died. From the seeds of common bent grass we could identify peritheciums of *Gibberella* sp.. Other fungal species occurred also were *Physarum nutans, Stemphylium botryosum, Torula graminicola, Gonatobotrys* sp. and *Phoma epicoccina.* On seeds of crested dog's tail we have found the above mentioned fungal species and beside there *Septoria* sp., *Ascochyta* sp., *Myrothecium gramineum*, *Acremoniella atra* and *Oedocephalum glomerulosum*. Both in Hungary and Austria the occurrence of *Ulocladium* sp. and *Pithomyces chartarum* identified from seeds of yellow oat grass and crested dog's tail, as well as *Culvularia lunata*, *Septonema* sp. and *Embellisia* sp. from seeds of crested dog's tail are considered new data.

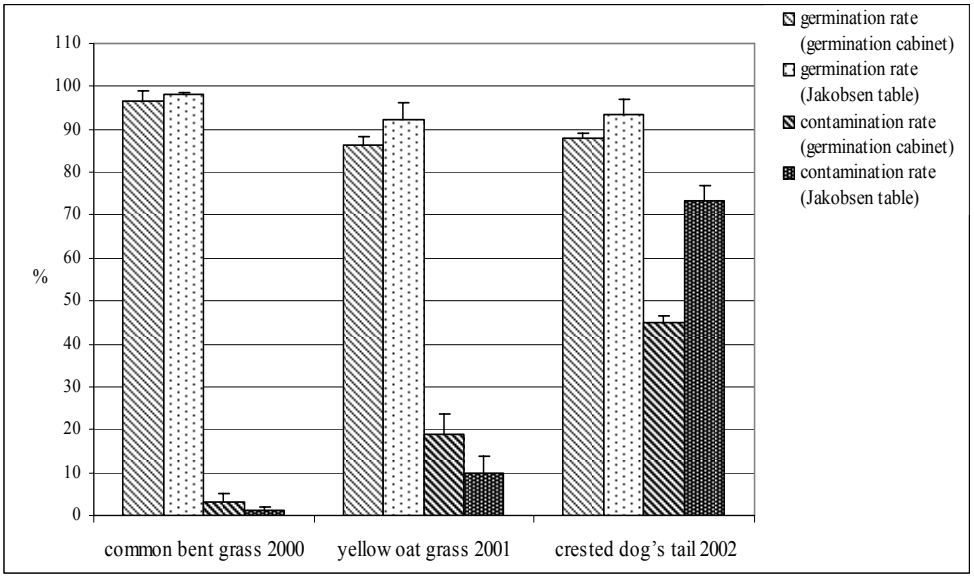

Figure 1. Germination and fungal contamination rate of the grass seeds of species tested at the first observation.
Note: on the 7th day in case of common bent grass and yellow oat grass, 10th day at crested dog's tail.

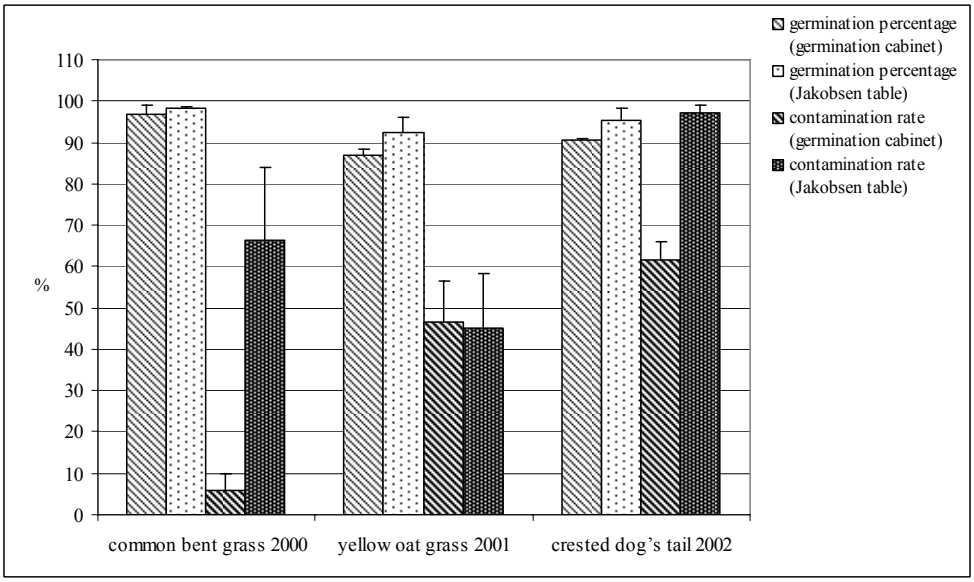

Figure 2. Germination and fungal contamination rate of the grass seeds of species tested at the 2nd observation.

Note: 21st day in the case of yellow oat grass and crested dog's tail, and 28th day at common bent grass.

Table 1. Rate of fungal genera and species found on seeds at the first observation (%)*

Fungal genera/species	common bent grass (*Agrostis capillaris*)		yellow oat grass (*Trisetum flavescens*)		crested dog's tail (*Cynosorus cristatus*)	
	Gc	J	Gc	J	Gc	J
Alternaria spp.	8,3	0,0	17,1	22,5	75,0	69,7
Cladosporium spp.	58,3	25,0	92,1	57,5	26,1	18,0
Stachybotrys atra	0,0	0,0	0,0	2,5	0,0	4,4
Epicoccum nigrum	8,33	0,0	0,0	12,5	1,1	24,8
Bipolaris, *Drechslera* spp.	0,0	25,0	0,0	0,0	5,0	5,1
Fusarium spp.	0,0	25,0	0,0	0,0	0,5	0,0

Note: Gc: germination cabinet, J: Jakobsen table

***:** 7th day at common bent grass and yellow oat grass, 10th day at crested dog's tail.

*Table 2.*Rate of fungal genera and species found on seeds at the 2nd observation (%)*

Fungal genera/species	common bent grass (*Agrostis capillaris*)		yellow oat grass (*Trisetum flavescens*)		crested dog's tail (*Cynosorus cristatus*)	
	Gc	J	Gc	J	Gc	J
Alternaria spp.	8,7	0,0	26,9	13,8	77,6	45,5
Cladosporium spp.	30,4	0,0	74,2	8,8	21,5	6,4
Stachybotrys atra	21,7	98,4	13,4	79,0	3,6	74,5
Epicoccum nigrum	0,0	0,0	0,0	3,8	1,2	14,6
Bipolaris, *Drechslera* spp.	21,7	0,0	0,0	0,4	1,2	2,6
Fusarium spp.	13,0	0,4	0,0	0,0	1,6	0,0

Note: Gc: germination cabinet, J: Jakobsen table

***:** 21st day at yellow oat grass and crested dog's tail, and 28th day at common bent grass.

The correlation calculation of the data show, that there was no correlation between the germination rate and fungal contamination rate (r_l= -0,80,

r_2= 0,37), that means that the fungal contamination of the seeds did not have an effect on the germination rate. However, some of the identified pathogens (like *Alternaria* spp., *Fusarium* spp., *Bipolaris* spp., *Drechslera* spp. and *Stemphylium* sp.) may cause seedling killing, may hinder the development of young plant. Also the occurrence of the large-scale presence of the seed contaminating throphic fungi, or rather weakening parasites may result in a weaker germination (Neergaard 1979), or they may even damage the developed plants (Eken et al. 2006).

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COMPARATIVE SEED PATHOLOGICAL INVESTIGATIONS ON CULTIVATED GRASS SPECIES

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The authors investigated comparative seed pathology of the species of common bent-grass, (*Agrostis capillaris* L), yellow oat-grass (*Trisetum flavescens* L.) and the crested dog's-tail (*Cynosorus cristatus* L.) using two different methods. Besides the mapping of fungal species occurring on seeds of the grass species tested, they also investigated effect of fungal contamination on the germination rate of the seeds. They identified from seeds of the three grass species 21 fungal genera belonging to the *Deuteromycota*. Among them the saprotrophic *Alternaria, Epicoccum, Cladosporium* and *Stachybotrys* proved to be dominant. They identified in many cases also the plant pathogenic genera of *Bipolaris, Drechslera, Fusarium, Septoria,* and *Ascochyta*. The occurrence of *Curvularia, Ulocladium, Embellisia, Septonema, Pithomyces* genera from seeds of crested dog's-tail, and *Ulocladium* and *Pithomyces* from seeds of yellow oat grass are considered new data.

INDUCTION OF DISEASE RESISTANCE BY SALICYLIC ACID, SODIUM BENZOATE AND POTASSIUM MONOPHOSPHATE AGAINST *USTILAGO MAYDIS* IN MAIZE PLANTS

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Plants can be induced locally and systemically to become resistant to disease through various biotic or abiotic stresses. Pretratment of susceptible plants with avirulant pathogens (biotic inducers) or certain chemicals (abiotic inducers) such as salicylic acid (SA) can enhance resistance to subsequent attack, not only at the site of treatment, but also in tissues distant from the initial treatment sites. Cohen (2002) added that biotic and abiotic agents may induce resistance in plants against pathogens. Abiotic agents may be synthetic or natural ones.

As an alternative to fungicide application, it may be possible to utilize a scheme for inducing plant defences in order to provide protection against pathogens. Such an induced defence response known as a systemic acquired resistance (SAR) could provide a sufficient protection against pathogens (Spletzer and Enyedi, 1999). They also added that challenge inoculation of 200 mM SA treated tomato (root feeded) using conidia of *Alternaria solani* resulted 83% fewer lesions per leaf and 77% reduction in blight leaf area compared to control (plants did not received SA.). The cited data indicate that root feeding of 200 mM SA to tomato plants can (i) significantly elevated foliar SA levels, (ii) induced PR-IB gene expression, and (iii) activated SAR that was effective against *A. solani*. White (1979) showed that exogenous application of SA and certain other benzoic acid derivatives induce rsistance to TMV virus and accumulation of pathogenesis related (PR) proteins. Yalpani et al. (1991) and Klessing and Malamy (1993) added that level of endogenous SA had been closely correlated to the induction of PR-proteins. Moreover, exogenous application of SA induces the same sets of SAR genes that were expressed following biological SAR induction. These observations had led to the idea that SA could be an endogenous signal responsible for triggering resistance (Ward et al. 1991; Uknes et al. 1992, 1993). Many researchers supported these observations i.e. Rasmussen et al. (1991), Shulaev et al. (1995), Chasan (1995) and Molders

et al. (1996) who showed that SA accumulation was essential for the development of SAR. It had been postulated that SA was the phloem-mobile long distance signal that travels through the plant and induces systemic resistance. Morris et al. (1998) added that both of SA and benzothiadiazole-7carbothioic acid (BTH) were capable inducing the expressions PR_1 and PRs genes in maize. Treatment by BTH at 1 g/kg of corn seeds reduced the incidence of downy mildew disease from 39% to 17%, this induced level of protection was similar to the genetic resistance. Moreover Ward et al. (1991) showed that evocation of SAR response causes rapid accumulation of several PR proteins both in the intra- and extracellular regions of the leaf. Metraux et al. (1991) and Vernooij et al. (1995) added that expression of PR genes and the induction of SAR can occure in the absence of pathogens by way of the application of synthetic compounds such as 2,6 dichloroisonicotinic acid (DNA) and BTH. Ward et al. (1991) and Palva et al. (1994) also added that exogenous application of SA to plants can effectively induce SAR.

Kumar et al. (2003) reviewed that field experiments on six resistance inducer chemicals, benzoic acid was better than other chemicals in reducing stem rot disease incidence and severity in rice (caused by *Sclerotium oryze*) followed by naphthalene acetic acid and SA. Elad (1992) tested 18 free radical scavengers (antioxidants) for their ability to control grey mould (caused by *Botrytis cinerea*) and white mould (*Sclerotinia sclerotiorum*) in various crops. Most of the compounds significantly reduced the disease levels in at least one on the tested hosts, i.e. tomato, pepper, bean and rose. Tannic acid and ascorbic acid controlled grey mould of tomato fruits, benzoic acid improved the control activity on tomato leaves. Kataria et al. (1997) showed that among 16 chemicals known as resistance inducers (antioxidants), ascorbic acid and benzoic acid efficiently controlled both pre- and post-emergence damping off in *Phaseolus vulgaries* caused by *Rhizoctonia solani*. El-Ganaieny et al. (2002) added that using of salicylic acid against three *Fusarium* spp., viz. *F. oxysporum*, *F. moniliforme* (= *F. verticillioides*) and *F. solani* which causes damping off and basal rot of onion was more effective when applied as seed and transplant treatment than when applied as soil treatment under greenhouse conditions.

The aim of the present work is to study the effect of three inducer chemicals, viz. salicylic acid, sodium benzoate and potassium monophosphate on induction of maize common smut disease resistance using two induction periods and two application methods.

Materials and Methods

I. Effect of induction period on resistance of common smut under greenhouse conditions

Maize cv. Balady were sown in pots 30 cm in diameter each contaning 8 kg soil/pot by 15 seeds/pot. Three pots were used for each treatment as repetition. After 10 days of sowing, the emerged seedlings were thined to 10 plants/pot. Three inducer chemicals viz. salicylic acid (SA), sodium benzoate (BA), potassium monophosphate (KH_2PO_4) at concentration 10 mM were used. After 15 days from sowing the upper and lower surfaces of the leaves were smeared by each of the inducer chemicals separetly by helping a piece of cotton. The treated plants were left for two different induction periods, 8 and 16 days respectively, and then inoculted by *Ustilago maydis*. Seedlings which were inoculted only by *U. maydis* were used as control. All treatments were kept under natural conditions during July and August 2004. The same expriment was repeated and all treatment were also kept under the natural condition during July and August 2005. After 30 days of inoculation by *U. maydis*, disease incidence was estimated as percentage of diseased plants (infection %) and disease severity which were expressed as disease index (DI). Disease index was estimated according to the size and numbers of galls which were classified into 8 classes: 0 = no infection, 1 = galls less than 1 cm in diameter, 2 = galls ranged from 1 to 2 cm in diameter, 3 = ranged from 2 to 3 cm in diameter, 4 = ranged from 3 to 4 cm in diameter, 5 = from 4 to 5 cm in diameter, 6 = from 5 to 6 cm in diameter, 7 = ranged from 6 to 7 cm in diameter, 8 = from 7 to more than 8 cm in diameter. Consequently, the following equation was set for disease index estimation:

$$DI = \frac{\sum(NPC \times CR)}{NIP \times MSC} \times 100$$

Where: NPC = Number of plants in class rate
CR = class rate
NIP = Number of inoculted plants
MSC = Maximum severity class rate

Fresh weight (g) and plant height (cm) were also measured.

II.- Effect of smearing and injection of maize leaves by inducer chemicals on resistance of common smut disease under greenhouse conditions

Maize cv. Balady was shown in pots as mentioned above. The same inducer chemicals which were used as in the formerly mentioned experiment in two different concentrations, 10 and 20 mM. After 18 days of sowing, upper and lower surfaces of leaves were separetly smeared and injected in the midvein by 1 ml of the two concentrations of the three inducer chemicals. After 16 days of treatments, the seedlings were inoculated by *U. maydis*. Seedlings inoculated by *U. maydis* were used as control. Three pots each containing 8-10 seedlings were used as replicates. Disease severity was estimated as mentioned earlier. The experiment was performed during July and August in two successive seasons of 2005 and 2006 under greenhouse conditions.

Results

I. Effect of induction periods of three inducer chemicals on disease incidence and disease severity of common smut disease of maize plants under greenhouse conditions

Data presented in Table 1 and 2 showed that no significant differences has been observed between the two induction periods 8 and 16 days, using the three tested inducer chemicals viz. SA, BA and KH_2PO_4 on disease incidence of common smut disease of maize plants. The most effective treatment on the disease incidence (infection %) were BA (9.3%) and KH_2PO_4 (6.7%) compared to control (21.7%) after 16 days induction period in the first season, and KH_2PO_4 (29.3%) compared to control (63.7%) in the second one. On the other hand, a significant difference was found between the two induction periods, 8 and 16 days using the three inducer chemicals on disease severity (DI %) in the two testes seasons with an exception of SA after induction period of 8 days and KH_2PO_4 after 16 days. The most effective inducer chemicals on disease severity (DI %) were BA (6.34%) and KH_2PO_4 (5.83%) compared to control (13.28%) after 16 days induction period in the first season and BA (15.27%) and KH_2PO_4 (15.33%) in comparison with control (42.85%) after 8 days induction period in the second one.

Table 1. Effect of induction periods of three inducer chemicals on disease incidence (infection %) and disease severity (DI %) of common smut disease of maize plants (cv. Balady) under greenhouse conditions during 2004 season

Inducer chemicals	Induction period (days)	Disease incidence (infection %)	Disease severity (DI %)
SA	8	15.3 ^{abc}	10.12^{c}
	16	22.7^{c}	15.93^{e}
BA	8	12.0^{ab}	9.37^{b}
	16	9.3^{ab}	6.34^{a}
KH_2PO_4	8	18.3^{bc}	12.87^{d}
	16	6.7^{a}	5.83^{a}
Control	8	21.7^{c}	13.28^{d}
	16	21.7^{c}	13.28^{d}

Table 2. Effect of induction periods of three inducer chemicals on disease incidence (infection %) and disease severity (DI %) of common smut disease of maize plants (cv. Balady) under greenhouse conditions during 2005 season

Inducer chemicals	Induction period (days)	Disease incidence (infection %)	Disease severity (DI %)
SA	8	29.0^{a}	22.3^{c}
	16	36.0^{a}	22,7^{c}
BA	8	40.7^{ab}	15.27^{a}
	16	58.3^{bc}	28.13^{d}
KH_2PO_4	8	20.7^{a}	15.33^{a}
	16	29.3^{a}	18.51^{b}
Control	8	63.7^{c}	42.85^{e}
	16	63.7^{c}	42.85^{e}

II. Effect of leaf smearing of three inducer chemicals on disease incidence and disease severity of common smut disease of maize plants as well as on fresh weight and plant height under greenhouse conditions

Data in Table 3 and 4 illustrated a positive effect on decreasing common smut disease by smearing leaves with the inducer chemicals SA, BA and KH_2PO_4 at two different concentrations 10 and 20 mM after 16 days incubation period. All tested inducer chemicals significantly decreased disease incidence and disease severity compared to control in the two tested seasons with an exception of BA at 10 mM and KH_2PO_4 at 20 mM in the first season. The most effective inducer chemicals were SA and BA at 20 mM. All inducer chemicals showed an increase in the fresh weight (g) during the two season and plant hight in the second season compared to control.

Table 3. Effect of leaf smearing of three inducer chemicals at two different concentrations on disease incidence and disease severity of common smut disease of maize plants (cv. Balady) as well as on fresh weight and plant height under greenhouse conditions during 2005 season

Inducer chemi-cals	Conc. (mM)	Disease incidence (infection %)	Disease severity (DI %)	Fresh weight (g)	Plant height (cm)
SA	10	36.30^{b}	22.68^{c}	351.7^{e}	95.3^{ab}
	20	25.00^{ab}	17.50^{b}	421.7^{f}	111.7^{b}
BA	10	58.30^{c}	28.13^{d}	266.7^{b}	105.0^{ab}
	20	15.00^{a}	5.10^{a}	348.3^{e}	105.7^{ab}
KH_2PO_4	10	29.30^{ab}	18.51^{b}	298.0^{c}	93.3^{ab}
	20	35.30^{b}	27.38^{d}	333.3^{d}	10.3^{ab}
Control		63.70^{c}	42.85^{e}	231.7^{a}	69.3^{a}

Table 4. Effect of leaf smearing of three inducer chemicals at two different concentrations on disease incidence and disease severity of common smut disease of maize plants (cv. Balady) as well as on fresh weight and plant height under greenhouse conditions during season 2006

Inducer chemi-cals	Conc. (mM)	Disease incidence (infection %)	Disease severity (DI %)	Fresh weight (g)	Plant height (cm)
SA	10	08.66[a]	04.76[a]	250.84[c]	79.86[bc]
	20	08.67[a]	13.63[c]	255.60[c]	85.70[c]
BA	10	19.66[b]	18.75[d]	293.50[e]	84.36[c]
	20	09.66[a]	06.66[b]	285.74[e]	88.90[c]
KH_2PO_4	10	19.17[b]	13.46[c]	276.17[d]	73.33[bc]
	20	39.66[c]	18.15[d]	248.26[c]	73.70 [bc]
Control		63.08[d]	43.01[e]	201.3 [a]	56.80[a]

III. Effect of leaf injection of three inducer chemicals on disease incidence and disease severity of common smut disease of maize plants as well as on fresh weight and plant height under greenhouse conditions

Data presented in table 5 and 6 showed that the injection of SA and BA into midvein of maize seedlings leaves significantly decreased the common smut disease by decreasing the gall size (DI %) in both tested years and the disease incidence (infection %) in the second year. All inducer chemicals showed increase in the fresh weight (g) and plant hight both in tested years compared to control. Generally, the three used inducer chemicals increased the fresh weight and plant hight.

Table 5. Effect of leaf injection of three inducer chemicals at two different concentrations on disease incidence and disease severity of common smut disease of maize plants (cv. Balady) as well as on fresh weight and plant height under greenhouse conditions during 2005 season

Inducer chemi-cals	Conc. (mM)	Disease incidence (infection %)	Disease severity (DI %)	Fresh weight (g)	Plant height (cm)
SA	10	$36{,}00^{ab}$	23.43^{c}	431^{c}	117.3^{c}
	20	38.0^{ab}	19.79^{b}	318.3^{b}	99.0^{b}
BA	10	61.0^{bc}	28.75^{d}	463.7^{c}	121.0^{c}
	20	21.0^{a}	15.78^{a}	344.0^{b}	98.7^{b}
KH_2PO_4	10	64.76^{c}	52.17^{f}	550.3^{b}	93.0^{b}
	20	53.0^{abc}	30.0^{d}	316.3^{b}	95.3^{b}
Control		63.7^{bc}	42.85^{e}	249.7^{a}	69.3^{a}

Table 6. Effect of leaf injection of three inducer chemicals at two different concentrations on disease incidence and disease severity of common smut disease of maize plants (cv. Balady) as well as on fresh weight and plant height under greenhouse conditions during 2006 season

Inducer chemi-cals	Conc. (mM)	Disease incidence (infection %)	Disease severity (DI %)	Fresh weight (g)	Plant height (cm)
SA	10	45.86^{b}	25.00^{c}	250.6^{d}	76.40^{ab}
	20	35.00^{a}	23.55^{ab}	221.2^{c}	75.80^{ab}
BA	10	46.67^{b}	34.85^{d}	207.73^{b}	81.86^{ab}
	20	43.33^{ab}	25.00^{c}	220.60^{c}	84.13^{b}
KH_2PO_4	10	35.49^{a}	22.59^{a}	213.51^{b}	83.0^{ab}
	20	35.54^{a}	24.10^{bc}	200.63^{b}	91.66^{b}
Control		67.67^{c}	43.01^{e}	201.56^{b}	56.80^{a}

Discussion

Induced resistance has been described in a wide variety both of dicots and monocots. Under our test conditions leaf smearing by SA, BA and KH_2PO_4 decreased the infection and disease index percentage significantly compared to control in the two tested years, 2005 and 2006. The inducer

chemicals increased the fresh weight and plant height. Injection of inducer chemicals into midvein of maize seedling leaves decreased the common smut disease by decreasing the gall size (disease index). Such inducer chemicals did not were phytotoxic to maize seedlings and restricted *U. maydis* infection, indicating that the three compunds may act synergistic. Thus resistance inducers can achieve a significant reduction of common smut disease incidence.

Our results showed also, that pretreatments of maize by SA, BA, and KH_2PO_4 respectively, 16 days before pathogen infection was much better in reducing *U. maydis* infection development than 8 days before. These results are similar to those obtained by Malolepsza (2005) who showed that the pretreatments of tomato with BTH and HER and cucumber with SA, BTH and INA before pathogen infections (24 hrs) reduced *Botrytis cinerea* and *Colletotricum lagenarium* (= *C. orbiculare*, Kövics, 2000) infection development in tomato and cucumber at 6 and 12 hrs, respectively.

The present results could be interpreted by those obtained by White (1979) who showed that exogenous application of BA (benzoic acid) derivatives induces resistance to TMV and accumulate PR proteins. Klessing and Malony (1993) added that SA could be an endogenous signal responsible for triggering resistance and closely correlated to the induction of PR proteins. Rasmussen et al. (1991), Shulaev et al. (1995), Chasen (1995) and Molders et al. (1996) also showed that SA accumulation was essential for the development of SAR. It had been postulated that SA was the phloem-mobile long distance signal that travels through the plant and induces systemic resistance. Moreover, Elad (1992) and Morris et al. (1998) reported that application of SA reduced the early blight in tomato, root rot in cowpea, bacterial blight in cotton, grey mould in various crops (tomato, pepper and bean) and anthracnose in maize.

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INDUCTION OF DISEASE RESISTANCE BY SALICYLIC ACID, SODIUM BENZOATE AND POTASSIUM MONOPHOSPHATE AGAINST *USTILAGO MAYDIS* IN MAIZE PLANTS

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An attempt was made to study the effect of three inducer chemicals, salicylic acid, sodium benzoate and potassium monophosphate on induction of disease resistance against *Ustilago maydis* causing common smut disease of maize plants. Two induction periods, 8 and 16 days, as well as two application methods, viz. leaf smearing and leaf injection, were studied for the three inducer chemicals. No significant effect has been observed between the two induction periods using the three inducer chemicals on disease incidence (infection %) of common smut. On the other hand, a significant difference was found among the two induction periods using the three inducer chemicals on disease severity (DI %). In general, the three inducer chemicals decreased the disease incidence and disease severity using both induction periods. As to the two application methods, leaf smearing and leaf injection, a significant decrease in disease incidence and disease severity of common smut disease as well as significant increase in plant weight and height was observed using the three inducer chemicals by both methods of application.

A NEW, RAPID AND NON-DESTRUCTIVE BIOPHYSICAL METHOD (CHLOROPHYLL *A* FLUORESCENCE) PROVES THAT GROWTH PROMOTING ENDOPHYTES ALLEVIATE CD STRESS IN *CICER ARIETINUM* L.

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Introduction

In India, the concentration of cadmium (Cd) into the soil is increasing day by day, due to the application of chemical fertilizers especially phosphate fertilizers. This is one of the major problems for the Indian farmers. Cadmium exerts a toxic effect to the plant and results in leaf chlorosis and necrosis with some of the morphological changes like decrease in growth in size and tissues (Becerril et al., 2002). In plants, Cd toxicity indirectly affects the photosynthetic apparatus of the leaf and can be evaluated by analysis of chlorophyll *a* fluorescence. Chlorophyll *a* is used as a rapid and non-destructive biomarker for the assessment of stress caused by different environmental factors (Srivastava et al., 1999). The availability of Cd to plants and, thus its toxicity depends on complex rhizospheric reactions, involving not only exchange processes between soil and plants but also microbial activities. In this respect, mycorrhizal fungi appear to play a central modulating role.
To overcome all these problems and to protect the plant against toxic metals, arbuscular-vesicular mycorrhiza fungi (AMF) and *Piriformospora indica* may play a promising role due to their growth promoting characteristics.

Mycorrhizae (fungus root) enter into a mutualistic relationship with plant roots, in which the fungi actually become integrated into the physical structure of the roots. The fungus derives nutritional uptake from the plant roots, without causing any plant disease.
Chickpea (*Cicer arietinum* L.) is an ancient crop of India which supplies 70 to 90 % of the worlds chickpea. It can grow in low rain fall and poor soils.

The main aim of the present study was to analyze and evaluate the cadmium-stress tolerance of chickpea after colonization of roots by *Piriformospora indica, Glomus mosseae* and *G. caledonium.*

Materials and Methods

Photobionts

Cicer arietinum cv. Chafa

Mycobionts

(i) *Piriformospora indica*, (ii) *Glomus mosseae*, *(*iii) *G. caledonium*

Culture medium for *Piriformospora indica*
P. indica was grown on Kaefer's medium (Kaefer, 1977).

Multiplication of inocula of *Glomus caledonium* and *G. mosseae*

The multiplication of AMF was carried out in *Zea mays.* The maize seeds were sown in sterile soil. The pot mixture (soil:sand in 3:1) was sterilized three times to inactivate the spores which were already present in the soil. After 3 months, inocula were assessed for colonization and preserved for further studies. The used inocula were *Piriformospora indica, Glomus mosseae* and *G. caledonium.* Proper controls were also maintained.
The seeds of two varieties of chickpea were obtained from Dr. Panjabrao Deshmukh Krishi Vidyapith, Akola, Maharashtra state, India. The healthy seeds of chickpea were surface sterilized by treating with 0.1% $HgCl_2$ and water for 2 minutes. Then kept overnight at room temperature for incubation in sterile distilled water. These seeds were ready for sowing.
Eighteen pots were filled with 3 kg of pot culture (soil:sand in 3:1) with 1-2 cm layer of mycorrhizal culture. Proper controls (without AMF) were also maintained. Twelve pots were used for chickpea Chafa including a control (only with Cd without cultures and without Cd and culture). In each pot, 3 plants were grown. The experiments were carried out in triplicate.
The surface sterilized seeds were sown on first day of October 2004. The seeds were germinated on the third day at the temperature 29-30^{o}C. Fluorescence were measured periodically at the interval of 7 days by using Handy PEA.
Cadmium stress was induced to each plant after 14 days from cultivation, excluding control. The initial concentration of cadmium was 10 μg/g of soil. At the interval of fifth day, the concentration of cadmium was increased by

10 μg, so that the stress was increased by 10 μg Cd/g of soil. The same procedure was continued up to November 2 and the stress was increased upto 40μg/g of soil. But phenotypically the plants did not show any significant change. So the stress (Cd concentration) was increased from 10μg to 100 μg/g of soil by adding 60μg Cd/g of soil. On every 5^{th} day stress was increased by 100 μg upto the 400 μg/g of soil.

Measurement of Chlorophyll *a* fluorescence

Handy PEA (Plant Efficiency Analyzer, from Hansatech Instruments Pvt. Ltd, UK) is a fluorimeter which has high resolution. The measurement process starts with the placement of lightweight plastic leaf clips at the measuring sites. The leaf clip shutter blade was closed to prevent the entry of light and the clip left in place for several minutes to provide dark adaptation.

During the measurement, PEA sensor unit was attached to the clip. The shutter blade of the clip can then slide back to expose the dark-adapted leaf to the sensor unit. This contains both a custom array of red light-emitting diodes (LEDs) providing saturating light levels for accurate F_m determination, and a fast response, low noise fluorescence detector for accurate determination of F_o.

On completion of a measurement the parameters F_o, F_m, F_v and F_v/F_m are automatically calculated from the recorded data. These parameters or the original data then transferred in a computer for further analysis. The data then analyzed by using a computer software program, Biolyzer and can be viewed on the display before deciding whether to retain or discard them. This flexible data storage system allows the user to decide on the most appropriate utilization of available memory.

To stain the roots the method suggested by Phillips and Hayman (1970) was followed and for assessment of colonization, the slide method proposed by Giovanneti and Mosse (1980) was applied.

Statistical Analysis

To analyze whether there is any significant difference between the performance of each culture with the control, we used ‘one way ANOVA’ for the relative variability within the separate classes of the experiment.

Results and Discussion

The endophytes *Piriformospora indica, Glomus caledonium* and *G. mosseae* improve the growth and biomass production of chickpea**.** These endophytes colonized roots of the host plant (Figure 1D, E, F).

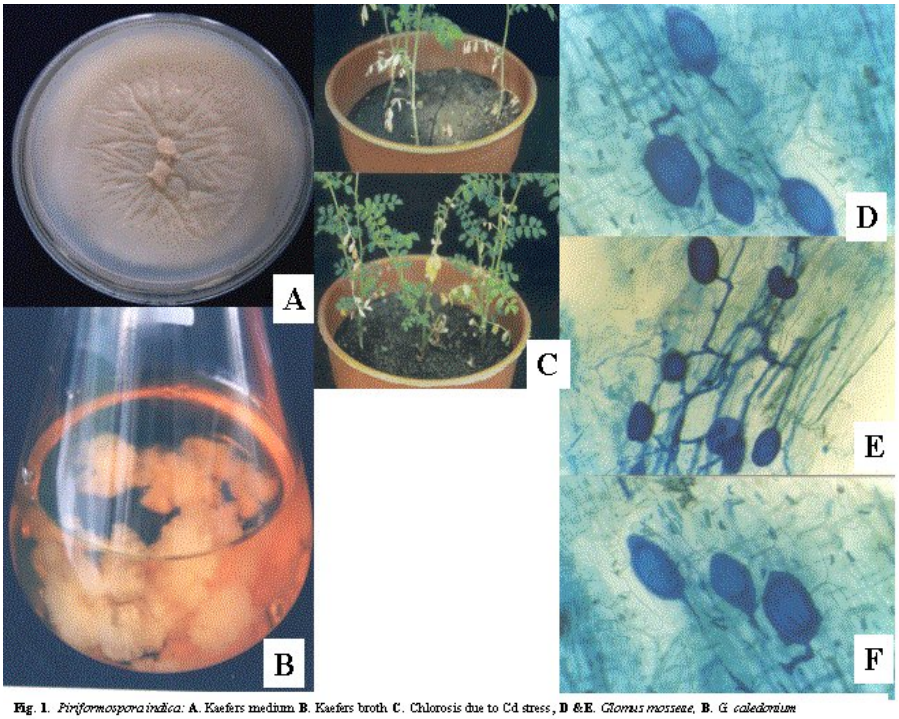

Fig. 1. *Piriformospora indica:* **A.** Kaefers medium **B.** Kaefers broth **C.** Chlorosis due to Cd stress, **D &E.** *Glomus mosseae*, **B.** *G. caledonium*

The possible benefits include increased crop yield, protection against stress condition by increasing tolerance to heavy metal and against pathogen and leaf diseases upto a certain level. Spores of the endophytes were used for the growth promotion of chickpea after giving Cd stress. *P. indica* showed the maximum performing index output in chickpea as compared to *G. caledonium* and *G. mosseae*. This demonstrates that cd treated plants are in stress and endophytes helps in mitigation of effect of Cd.

The percentage of colonization and height of the plants were also improved as compared to control and Cd treated plants (Table 1). Cadmium is a heavy metal and exerted toxic effect to these plants, resulted in chlorosis and necrosis (Fig 1C). At low concentration the plants did not show the significant phenotypic changes but physiological changes were observed. When the concentration of cadmium was increased upto 100 μg/g of soil, it exerted its toxic effects and have shown the symptoms like yellowing of leaves. These symptoms were observed much greater in Cd treated plants than the Cd + *P. indica* , Cd + *G. caledonium* and Cd + *G. mosseae* treated plants.

Table 1. Percentage colonization and height of Cd treated plants after inoculation with different endophytes

Treatment	%Colonization	Height (in cm)
Control	No	19.00 (±3.80)
Cd treated	No	14.25 (±1.97)
Cd + Gc	75.0 % (±1.52)	28.12 (±6.38)
Cd + Gm	70.0 % (±2.08)	24.12 (±4.56)
Cd + Pi	85.0 % (±1.63)	26.00 (±5.70)

All values are mean ± S.D. Mean values are significantly different at $P<0.05$

When the Cd concentration was increased upto 200 μg/g of soil, the branches of Cd treated plants turned to brown. But the branches of the Cd + endophytes treated plants did not turn brown. Later, after increasing the concentration (300 μg/g of soil) the chlorosis and necrosis took place. At this stage, the growth of Cd treated plants was stopped. The Cd treated plants of 'Chafa' variety were died at this stage.

The test endophytes – *Piriformospora indica*, *Glomus mosseae* and *Glomus caledonium* – promote the growth and increase the heavy metal tolerance in plants by forming symbiotic association with roots of plants. These endophytes also increases the nutrient uptake of the plant through the roots and photosynthetic activity with height and branching of the plants.

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A NEW, RAPID AND NON-DESTRUCTIVE BIOPHYSICAL METHOD (CHLOROPHYLL A FLUORESCENCE) PROVES THAT GROWTH PROMOTING ENDOPHYTES ALLEVIATE CD STRESS IN *CICER ARIETINUM* L.

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Summary

Cadmium stress was induced in *Cicer arietinum* (chickpea), which is the third important crop of India after wheat and rice. The measurements of chlorophyll *a* fluorescence were recorded after 10 days of inoculation with the help of Handy PEA (Plant Efficiency Analyzer). Cadmium stress was given to each plant after 14 days from sowing, excluding control (without stress) of each variety. The concentration of cadmium was increased after a period of 5 days by 10 μg to increase stress. We used endophytes, viz. *Piriformospora indica, Glomus caledonium* and *G. mosseae* to evaluate their effect on *Cicer arietinum* under Cd stress. The main aim of the present study was to assess the efficiency of endophytes on chickpea exposed to Cd stress. The tested endophytes promoted the growth, increased the heavy metal tolerance, the nutrient uptake, and photosynthetic activity with height and branching of the plants.

INFLUENCE OF CONSERVATION TILLAGE AND DIFFERENT NUTRIENT RATES ON THE LEAF DISEASES OF WINTER WHEAT

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In Hungary, the history of conservation tillage goes back to the beginning of the last century when Pethe, in 1818, constructed the "Hungarian plow-plant", which was used for seedbed preparation, seeding and harrowing, all in one operation. The advantages of the Hungarian plow-plant were elimination of trampling, higher yields and reduced consumption of time and energy. Conventional tillage dominated up to the end of the 1970s in Hungary. Introduction of new soil tillage systems was stimulated by reduced energy costs, soil protection, and a more economic soil moisture management.

Birkás et al (1989) gave an extensive survey of conventional and reduced tillage in Hungary, emphasizing that new methods of soil tillage were applied where they could result in lower production costs without any hazards to yields. (Butorac, 1994) Giving up conventional methods is necessary in order to improve moisture management, as well as to reduce dusting, CO_2 emission and organic matter reduction. (ECAF 1999, Birkás 2000, Gyuricza 2000)

Stringent measures of soil- and environment protection require less pesticide utilization, so agrotechnical and soil management possibilities will be of great importance. (Lehoczky és Percze, 2006)

Conservation tillage is practised on 45 million ha world-wide, predominantly in North and South America but its uptake is also increasing in South Africa, Australia and other semi-arid areas of the world. It is primarily used as a means to protect soils from erosion and compaction, to conserve moisture and reduce production costs. In Europe, the area cultivated using minimum tillage is increasing primarily in an effort to reduce production costs, but also as a way of preventing soil erosion and retain soil moisture. (Holland, 2004) The Soil Conservation Service, USDA, has predicted that as much as 95 % of United States cropland will be cultivated by conservation tillage by the year 2010. (Myers, 1983)

In Hungary, little is known about the effect of crop residues on the pathogens, probably the amount of residues left on soil surface is related to the severity of the disease.

Materials and Methods

Tillage treatments:

1. conventional tillage, ploughing as control (22-25 cm) (PL),
2. no-till (NT),
3. shallow cultivator use (14-16 cm) (SC),
4. cultivator use, leaving mulch (16-18 cm) (CLM),
5. disking (14-16 cm) (D),
6. loosening (35-40 cm) (L).

Plots have been fertilized on the 22nd of March 2006 at right angles to tillage treatments with four different nutrient rates in two replications, which meant 3x, 2x, 1x and 0x 34 kg/ha N. The assessment of the severity of leaf diseases was carried out on 31st of May 2006, in full flowering of winter wheat. The precipitation was 60 mm in March, 35 mm in April, 110 mm in May, totally 205 mm.

The assessment is applied during diffuse- or local type disease processes of resistance investigations, when the rate of infection is estimated. There are two methods for the evaluation of surface pathogen infection, relative and absolute evaluation. There are two types of absolute evaluation: with non-linear and linear scale.

The infection with appropriate intensity after spontaneous or provocated infection should be evaluated in optimal time. The infection rate is considered to be optimal, when the disease is developed, but the plants' senescence is not started yet.

At surface diseases, in order to determine the rate of infection, a 0-5 absolute evaluation scale is used with 0.5 units (0 = no infection, 5 = 100 % infection). The scale is linear, so the evaluation rate multiplied by 20, equals the infected surface % (for e.g. 1.5 = 30 %).

Results and discussion

Pathogens and symptoms of leaf spots

Leaf spots of winter wheat have come into the centre of interest in the last two decades. One reason is that pyrenophora tan spot damage is known since 1988 (septoria blotch was known before). The changes in the structure of agriculture had major roles, as well as the drastic reduction in fertilizer

application and heterogenity of the level of crop production. Extensive farming replaced intensive farming in many places. The scale of wheat monoculture and non-inversion tillage increased. These changes have clearly favoured necrotrophic fungi (pathogens of pyrenophora tan spot, septoria blotch and ear fusarium). (BASF, 2006)

Septoria leaf blotch can be caused by two pathogens: *Septoria tritici* Rob. ex Desmaz. and *Phaeosphaeria nodorum* (E. Müller) Hedjaroude. The anamorph form of the latter one is *Septoria nodorum* or as new scientific name is *Stagonospora nodorum* (Berk.) Castellani and Germano. Both species causes leaf spots mainly, but *Phaeosphaeria nodorum* infects the glumella of the ear as well, causing browning. *Septoria tritici* is more frequent in Hungary, but occasionally *Phaeosphaeria nodorum* occurs, too.

In the case of *Septoria tritici* leaf spots are long-shaped in the beginning, while at *S. nodorum* are oval. Later the spots are merged, in serious infection the leaves become dry. Identification of leaf symptoms with unaided eye is facilitated by the round, rough to the touch, black picnidia (sometimes perithecia) on the drying spots.

Pyrenophora tan spot is called as yellow spot, as well. The pathogen of this disease is *Pyrenophora tritici-repentis* (Died.) Drechs., anamorph form (conidial) is *Drechslera tritici-repentis* (Died.) Schoemaker, previously known as *Helminthosporium tritici-repentis* Died.

The typical initial symptom of pyrenophora on the leaf is the dark-brown dot and surrounding yellow blotch (the place of conidial infection), due to the spreading toxin of the fungus. The spots are getting larger, merged and brown, in the case of severe infection leaves become dry. (BASF, 2006)

Influence of different tillage treatments and nutrient rates on the leaf diseases of winter wheat

During the evaluation leaf spot (septoria and pyrenophora), powdery mildew, leaf rust and take-all was found. Highest infection rate was determined for septoria and pyrenophora leaf spot, powdery mildew and leaf rust was found rarely. Take-all was detected under direct-drilling only. Powdery mildew occurred in traces under 3x nutrient rates in all tillage treatments, except direct drilling. Leaf rust was detected only under 3x N rate under conventional ploughing and shallow cultivation. Due to bacterias, physiological changes have been detected on lower leaves of the plants.

Table 1. Averages of leaf spot rates (0-5 scale) under four different N rates

Tillage treatments	Average
Conventional ploughing (PL)	0.5
Disking (D)	0.9
No-till (NT)	1
Shallow cultivator (SC)	1.1
Cultivator leaving mulch (CLM)	1.6
Loosening (L)	1.8

Our results proved that burying (PL) is an effective means of destroying plant pathogens and thus, an important means of plant disease control. Today, crop producers are shifting to surface-tillage systems to offset the rapidly rising costs of fuels, labour and soil erosion. (Phillips et al., 1980)

According to the fact, that the investigation has been carried out after a 4-year winter wheat monoculture, the results are quite promising when adopting soil conversation tillage systems.

The aforementioned fact is proved by the leaf spot infection of 20 % under direct-drilling. The healthy state of winter wheat can be due to the favourable plant protection effect of catch-crops (pea, mustard and rye).

Table 2. Effect of tillage treatments and decreasing N rate on the average value of leaf spot rates

Tillage treatments and N rate	Average
PL, NT, SC, CLM, D, L **3x** rate	1
PL, NT, SC, CLM, D, L **2x** rate	1.06
PL, NT, SC, CLM, D, L **1x** rate	1.25
PL, NT, SC, CLM, D, L **0x** rate	1.4

We stated that with decreasing nutrient rate, the average value of leaf diseases was increasing. Nitrogen makes plants more toughen against necrotrophic pathogens (fusarium, pyrenophora, septoria). Nitrogen application enhances the juvenility of winter wheat (inhibits ethylene-synthesis, so the senescence of plant tissues) and keeps the plant juvenile longer. If plants are well-provided with N, they are protected to a certain degree against fusarium, pyrenophora and septoria leaf spots. (BASF, 2006) Leaf spot rates under decreasing N rate in the same tillage treatment did not show clear tendency, for e. g. under disking 3x N rate 0.5; 2x N rate 0.5; 1x N rate 2; 0x N rate 0.5 leaf spot rates were detected.

At 3x N rate septoria was dominant, with decreasing N dose pyrenophora occurrence increased, regarding the whole stand.

The results of leaf spot evaluation carried out in the long-term soil tillage experiment are remarkable for some reasons. It can be stated that in the rainy growing season of 2006, tillage treatments as well as different nutrient rates influenced the incidence rate of leaf spot diseases. This fact can not be generalized as our results presumably do not tally with others', which were carried out in other places under different circumstances and different plant protection- and soil conditions. This makes prediction of future disease problems difficult because observations from one area may not necessarily be applicable to others. Taking into consideration, that the investigation has been carried out after a 4-year winter wheat monoculture, the results are quite promising when adopting soil conversation tillage systems.

According to Bailey (1996), over the long term, leaf spot diseases of cereals may be more affected by local environmental conditions than by changes in tillage practice.

Acknowledgement

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INFLUENCE OF CONSERVATION TILLAGE AND DIFFERENT NUTRIENT RATES ON THE LEAF DISEASES OF WINTER WHEAT

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Summary

The aim of our research in 2006 is to investigate the effects of different conservation tillage methods and nutrient rates on the leaf diseases of winter wheat. The investigations are carried out on the long-term soil tillage experimental field in József-major, which was established in 2002. Tillage methods were as follows: 1. conventional tillage, ploughing as control (22-25 cm), 2. no-till, 3. cultivator use (14-16 cm), 4. cultivator use, leaving mulch (16-18 cm), 5. disking (14-16 cm), 6. loosening (35-40 cm). Plots have been fertilized at right angles to tillage treatments with four different nutrient rates in two replications, which meant 3x, 2x, 1x and 0x 34 kg/ha N.

In order to evaluate leaf disease infection, a 0-5 scale was used. The evaluation was carried out on the 31st of May 2006 in full flowering. Septoria and Pyrenophora infection was dominant, leaf rust was rarely detected. We stated that with decreasing nutrient rate, the rate of leaf diseases was increasing. At 3x N rate was 1, at 2x N rate 1.06, at 1x N rate 1.25 and at no N 1.4 average infection value was detected under different tillage methods. Taking into consideration, that the investigation has been carried out after a 4-year winter wheat monoculture, the results are quite promising when adopting soil conversation tillage systems.

This research is supported by the Hungarian National Scientific Foundation (OTKA-49.049 and F046.670), KLIMA-05 and NKFP-6/00079/2005 programs and SZIE GAK Kht. József-major Experimental and Training Farm.

FLAYING HEIGHT OF INSECTS CONNECTED WITH MOON PHASES USED THE LIGHT-TRAP CATCH DATA

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Great many researchers have been studying the question of the height at which insect's fly. A clarification of the issue from the point of view of plant protection prognosis assumes special significance if there is sufficient evidence to prove that the height of flight is affected by the phases of the Moon. El-Ziady (1957) believes in the likelihood of insects flying higher at the time of a full Moon, so the catch is lower at this time than at other Moon quarters. He backed his assumption by the catch results of a suction trap placed at a height of 10 m. Earlier Williams (1936) found lower catch at full Moon. He thought it was because of the smaller gathering distance or moonlight has a direct influence on activity and reduces the number of flying insects. After more decades, there is not any recognised answer to this question.

Williams (1939) who used light-traps placed at distances of 2 and 10 m from the surface of the ground was a forerunner in examining the vertical dispersion of nocturnal insects. Taylor and Brown (1972) have found that some species fly in greater numbers to a light-trap placed higher (12m), while others prefer traps at a lower height (1 m). Callahan et al. (1972) collected insects with 15 UV light-traps positioned at different levels going up to 320 m of a television tower. The highest number of moths was trapped at heights of 7 - 25 m. From 83 - 320 m, the dispersion was close on even, but a remarkably higher number of moths were trapped in zones between the red light signals. More than halves of the insects of the 35 families of 9 orders of insects trapped were *Helothis zea* Boddie specimens. The ratio of this species at 320 m was 82%, stunningly high. Sorry their results were not examined in relationship with Moon phases. Taylor et al. (1979) captured migratory moths with light-traps of an identical type placed at heights of 0.6 and 24.5 m respectively from ground surface in Kenya. The catch of the trap placed high was but a fraction of the one placed low. This proportion is 1:11.4 in the case of the turnip moth (*Scotia segetum* Schiff.).

Aly (1990a) operated two Robinson type light-traps. These traps were put in 1.5 m and 18.5 m height from the ground. He examined the success of light trapping of *Paederus alfierii* Koch (*Staphylinidae*:

Coleoptera) in connection with the four moon quarters. The catch of lower trap was better at the time of new moon than full moon, but the difference was not significant in the higher one. Aly (1990b) showed in the catch of *Gryllus domesticus* L. (*Gryllidae*: *Orthoptera*) on the 1.5 m height trap, the light-trap catch is higher at new moon than full moon in summer of 1983. This event can not be seen in the catch of 18.5 m high trap. Aly and Shafi (1991) did not notice significant difference at the different moon quarters in the catch of *Componotos maculatus aegyptiacus* Em (*Formicidae*: *Hymenoptera*) at 1.5 m height. The light-trap catch was more successful during fall months at full moon in 18.5 m height.

There were operated two Jermy-type light-traps by Vojnits and Voigt (1971) at Tarcal at experimental yard of Research Institute for Viticulture and Aenology. The light source was a 100 W normal electric bulb in both trap. One trap was put between to the grape line, the height was about 1.5 m and the other was at the end of the line in 2.5 m height. The distance was about 15 m between these traps. There were determined not only grape moths, but also other Microlepidoptera species. Generally, the lower trap caught more specimen as the higher one, but the European corn borer (*Ostrinia nubilalis* Hbn.) was caught in higher number by the upper trap.

There were three fractional light traps in operation between 1967 and 1969 in Kecskemét operated by Járfás. These traps were put in three different heights and there were separated in every hour. The light sources of fractional light-trap were three fluorescent lamps (F-33 type, 40 W). Their length was 120 cm, and they were above one another. Járfás published the catch of different levels of several species, but he did not examine the causes of differences (Járfás, 1979).

It was shown in a latter study (Bürgés et al., 2003) the specimen number of migrant moths is highest just at full moon in low trap, so their flight activity is high during this time. Bürgés (1997) published separately the Macrolepidoptera catch for each family using the data in lower and higher trap at Rezi. Most of species were caught in both traps, but the higher caught more number of insect as the lower one. The exception was only in case of *Geometridae* and *Notodontidae* families. Herczig and Bürgés (1981) operated two light-traps in a closed stand of chestnut and oak in the vicinity of the village of Rezi, in the mountain range of Keszthely. Both traps were outfitted with 125 W HGL bulbs. One of the traps was placed at a height of 2 m from ground surface, the other at a height of 10 m in the canopy of a chestnut tree. The two traps worked at a distance of 100 m from each other. The trap working at 10 m captivated four times as many migratory moths than the one operating at 2 m. The trap high up also caught species not breeding in the surroundings.

Materials and Methods

We could use the whole Microlepidoptera data of traps at Tarcal. We thank for these data to Zoltán Mészáros.

Earlier József Járfás gave the fractional trap data of Kecskemét-Katonatelep (between 1967 and 1969) to use in our corporate studies. There are data of fall webworm (*Hyphantria cunea* Drury), turnip moth (*Scotia segetum* Schiff.) and European corn borer (*Ostrinia nubilalis* Hbn.) in it hourly separated according to the levels (Table 1).

Table 1. The catch data of examined species at different levels of fractional light-trap in Kecskemét between 1967 and 1969

Species Levels	*Hyphantria cunea* Drury		*Scotia segetum* Schiff.		*Ostrinia nubilalis* Hbn.	
	Number	*%*	*Number*	*%*	*Number*	*%*
Lower	1439	45.41	2349	41.28	394	27.94
Median	1178	37.17	2045	35.93	544	38.58
Higher	552	17.42	1297	22.79	472	33.48
Together	3169	100.00	5691	100.00	1410	100.00
Hours	875		950		702	

We used the whole Macrolepidptera catch data traps at Rezi (in 1976, 1978 and 1979), but only those nights were examined when both traps were in operation.

We used in earlier study (Nowinszky, 2003) 30 phase angle groups, calculated 360 phase angle values of the full lunation. Now we made only 10 phase angle groups, because we had less light-trap catch data. There were 12 phase angle in every phase angle groups in former study. Now we had 36 ones, because we contracted three groups, but the notation was the same so we could compare with the former results. The notation of phase angle group with full moon (0º, or rather 360° ± 18°) is 0. There are group notations -3, -6, -9 and -12 from this one to new moon through the first quarter. There are group notations 3, 6, 9 and 12 from full moon to new moon through the last quarter. The phase angle group containing new moon is ±15. The first moon quarter belongs to -6 group and last moon quarter belongs to +6 one.

We took into consideration only those hourly data from Kecskemét light-trap during the examination, which had successful catch at least one

trap. The number of caught specimen on each level was calculated hourly as a percental value of the whole number of insect was caught in the three traps. The percental data of examined species were categorized hourly and for every level into the above mentioned phase angle groups, then they were summarized and averaged. The catch results were very similar in median and upper level so they were contracted and after it we made a comparison between these results and catch in the lower trap.

We worked up according to the same method the Microlepidoptera and Macrolepidoptera data caught by light-traps at Tarcal and Rezi. We made the examinations using the contracted data of all species and not with separated for each species, because of the relatively not too much catch. We used only those data, when both traps were in operation. We assigned the number of caught individuals to the phase angle group of that night. We summarized the number of trapped individuals belonging to each phase angle group. We calculated the percental rate of individual number of lower and higher trap. We illustrated the results in the same way in all cases.

Results and Discussion

There are shown the specimen rate of fall webworm (*Hyphantria cunea* Drury), turnip moth (*Scotia segetum* Schiff.) and European corn borer (*Ostrinia nubilalis* Hbn.), caught by the lower and higher light-traps, in Table 1, 2 and 3 connected with phases angle groups of Moon. The percentage of individuals of Microlepidoptera species, caught in lower and higher light-trap at Tarcal, is shown in Table 4. The percentage of individuals of Macrolepidoptera species, caught in lower and higher light-trap at Rezi, is shown in Table 5.

Our results, got from the data of Kecskemét light-trap, prove the proportion of caught samples both of fall webworm (*Hyphantria cunea* Drury) and turnip moth (*Scotia segetum* Schiff.) is the most equable at the lower and higher levels at the time of full moon. These species fly in high proportion to 121 and 360 cm levels during full moon, than at the time of other moon phases. The proportion of caught specimen of European corn borer (*Ostrinia nubilalis* Hbn.) is highest just at full moon time in lower and higher levels. One reason can be insects fly in the air above 360 cm this time, but it can be also supposed they fly in great number near the ground level. We can not decide from these data which reason is correct.

The proportion of Microlepidoptera individuals, caught by lower and higher light-traps at Tarcal, is the highest at new moon, but it is higher at full moon than at first and last quarter and the proportion of caught species number is also similar.

The proportion of Macrolepidoptera individuals, caught by lower and higher traps is highest in the last quarter, the lowest in first quarter and at full moon. The proportion of caught species shows similar but more strikingly marked picture.

Of course the behaviour of some Microlepidoptera and Macrolepidoptera species can differ from the results got from the summarised data. It would be very important to put into operation light-traps in different higher levels at some observing stations and during longer period. It can be taken into consideration during making the plant protection forecast if there would be a proof, the individuals of several species fly in higher number in various heights during the time of different moon phases.

Fig. 1
Percentages of the individuals of the fall webworm moth (Hyphantria cunea Drury) at the low and high levels of Jásfás-type light-trap in connection with the phases groups of the Moon (Kecskemét, 1967-1969)

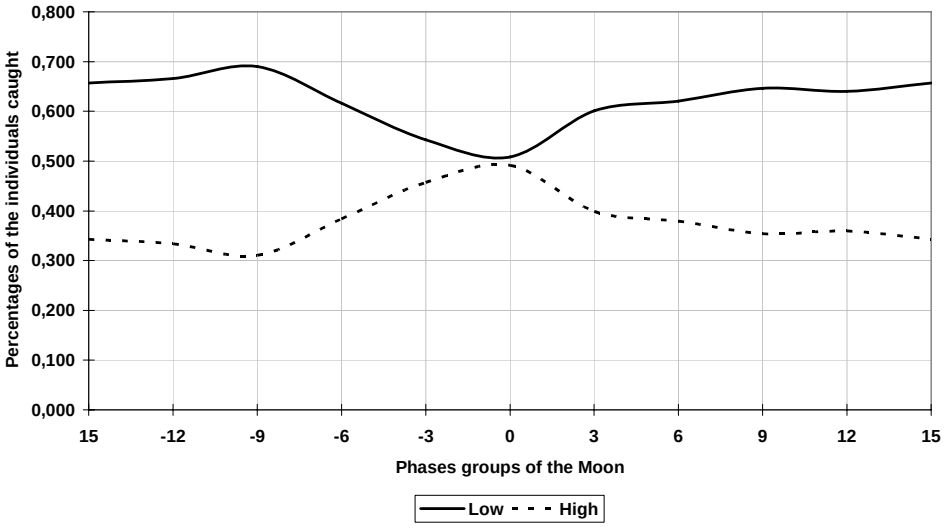


Fig. 2
Percentages of the individuals of turnip moth (Scotia segetum Schiff.) at low and high levels of Járfás-type light-trap in connection with the phases groups of the Moon (Kecskemét, 1967-1969)

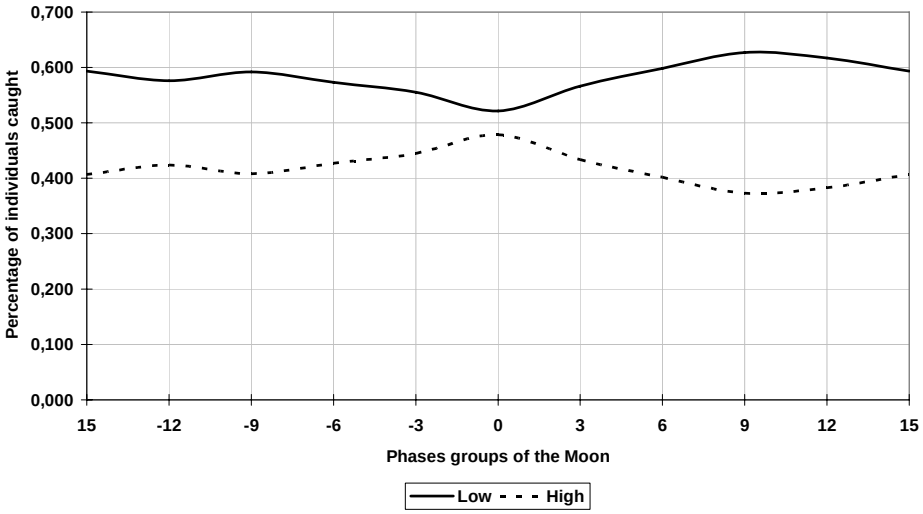


Fig. 3
Percentages of the individuals of European corn borer (Ostrinia nubilalis Hbn.) at the low and high levels of Járfás-type light-trap in connection with the phases groups of the Moon (Kecskemét, 1967-1969)

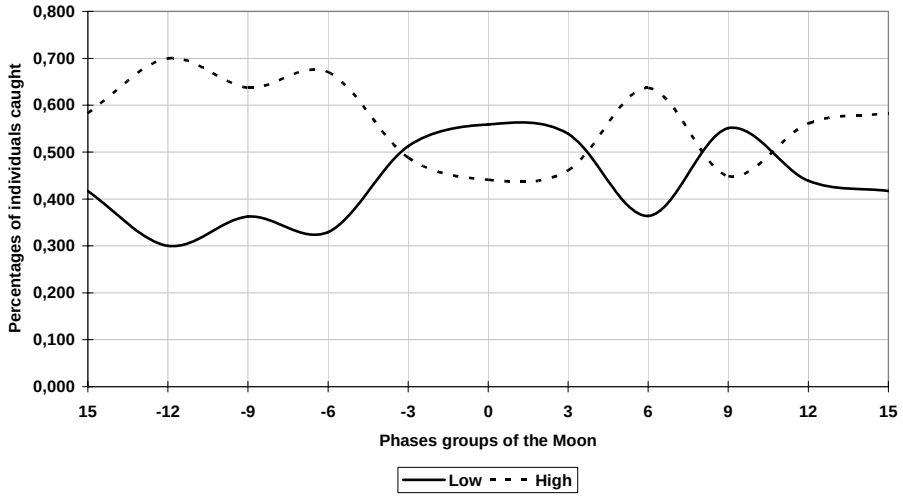


Fig. 4
Percentages of Microlepidoptera individuals caught at low and high light-traps in conection with the phases groups of the Moon (Tarcal, 1965-1968)

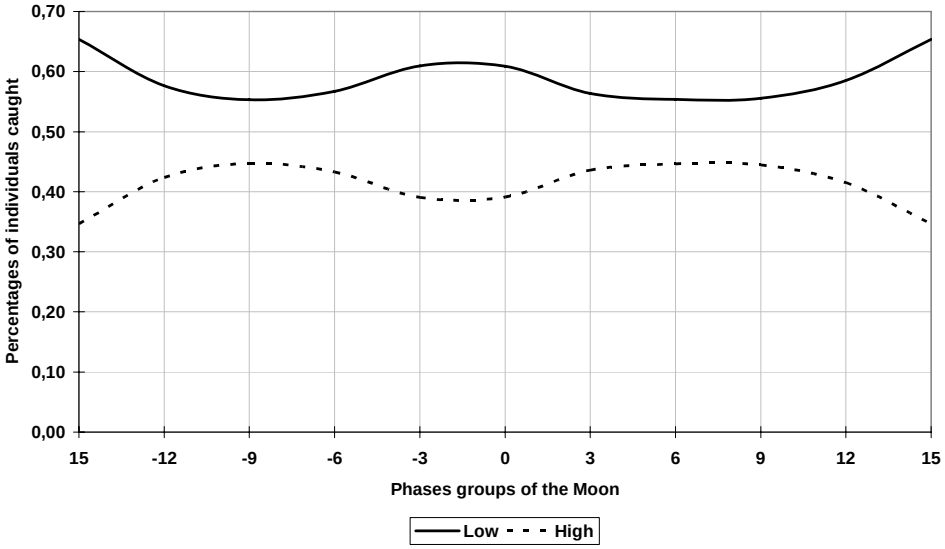
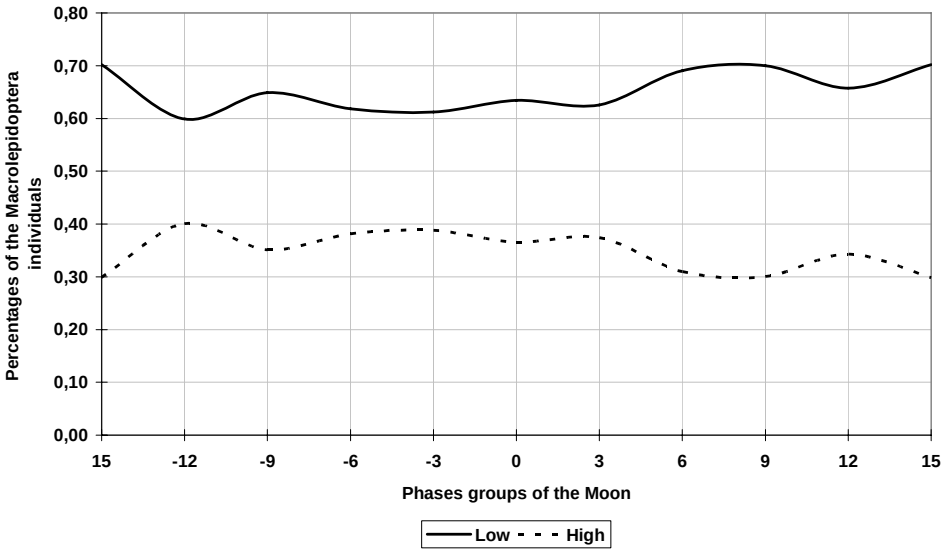


Fig. 5.
Percentages of Macrolepidoptera individuals at low and high light-traps in connection with the phases groups of the Moon (Rezi, 1976, 1978, 1979)



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FLAYING HEIGHT OF INSECTS CONNECTED WITH MOON PHASES USED THE LIGHT-TRAP CATCH DATA

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Summary

Great many researchers have been studying the question of the height at which insect's fly. A clarification of the issue from the point of view of plant protection prognosis assumes special significance if there is sufficient evidence to prove that the height of flight is affected by the phases of the Moon. We analysed light-trap data on Macrolepidoptera and Microlepidoptera species from three locations (Kecskemét, Tarcal and Rezi) in order to study the connection between moon phases and insect flight.

The effect of moon phases on the flying-height of studied taxa was proved. Of course the behaviour of some Microlepidoptera and Macrolepidoptera species can differ from the results got from the summarised data. It would be very important to put into operation light-traps in different higher levels at some observing stations and during longer period. It can be taken into consideration during making the plant protection forecast if there would be a proof, the individuals of several species fly in higher number in various heights during the time of different moon phases.

ROLE OF HEDGES IN PLANT PROTECTION

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The present agricultural practise is not sustainable and not efficient. It needs too much energy and material – as chemicals. It increases the environmental pollution and load; damages the soil, the living being – especially the benefitial organisms which consume pests.

Working out a sustainable, long term process the hedges can be used well. They give shelter to the pest's natural enemies and they have a windbreak function, they help the plants to keep humidity. The used chemicals can be cut. The environmental pollution can be reduced. The biotop-nets give migration way for plants and animals, and they develope the area's diversity.

In this work we examine the weight of grain to show the impact of hedges on yield of winter wheat.

INVESTIGATION ON THE EARLY COMPETITION BETWEEN YELLOW NUTSEDGE (*CYPERUS ESCULENTUS* L.) AND MAIZE

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Yellow nutsedge (Cyperus esculentus) is a cosmopolitan, tropical, subtropical plant. On the basis of Ujvárosi life-form it is a G_2 perennial plant, overwintering with tubers in the soil. Because of its rapid spreading by its extensive tuber- and seed production, yellow nutsedge is a strong competitor of the cultivated plants by causing severe yield losses. It causes severe yield losses of hoed crops. The importance of yellow nutsedge is characterized by the fact, that it was considered to be the 16th most important weed in the world in 1970. On the basis of EPPO IAS Panel at present this weed species is considered as one of the most harmful plant invaders of the world, due to its severe economic injury. It occurs in every continents.

Yellow nutsedge origins from the subtropical districts of North-Africa. It belongs to the Cyperaceae plant family. Botanists separate its cultivated type (*Cyperus esculentus* L. var. *sativus* (Boeck) from its four weedy variations. The occurrence of the weed variations are: *Cyperus esculentus* L. var esculentus in Southern Europe, Asia, and Northern America, *Cyperus esculentus* L. var *leptostachyus* (Boeck.) in Western Europe, North- and South-America, *Cyperus esculentus* L. var. *heermanni* (Kükenth.) and var. *macrostachyus* (Boeck.) in the United States and in the Netherlands (Dancza 1994).

The weedy variations are serious plant invaders in Europe, causing considerable damages in Europe only from the early 1970's. Their identification and investigation of its spreading is in progress from the early 1990's. Spontaneous occurrence of the weedy form of yellow nutsedge (Cyperus esculentus L. var. leptostachyus) were observed at first time by István Dancza in Hungary in August 1993 in the vicinity of Keszthely-Hévíz in maize crop on calcareous moor soil.

At present it occurs in four regions of Hungary, on the confines of 20 habitations. Somogy county is the most infected area, where it occurs on 10000 hectares. On 2500-3000 ha a constant control of this weed is now necessary.

Cyperus esculentus can be especially harmful in the spring sown thin-type root crops, mainly of maize, sunflower, potato and sugarbeets (Dancza et al. 2005).
According to Hunyadi and Almádi (1981) the most important interference between weeds and maize is the competition. The competition is an effort of two or more individuals to acquire the same factor in the same time. Competition can take place between different species (interspecific competition), within the species (intraspecific competition), between genotypes (intergenotypic competition) and within the genotype (intragenotypic competition). The maize plant, like any other plants, is sensitive to compete with weeds in its young phenological stage (Hunyadi et al., 2000). The aim of our investigation was to collect data on competition between maize and yellow nutsedge.

Materials and Methods

In the trials we used the substitution method. In these tests the two plant species are sown together, mixed in different rates, while the density of the mix is always the same. By this the behavior of a clean cropped species can be compared with the differently weed-mixed crops, and the mutual agressivity can be measured. Moreover the interspecific, and the intraspecific competition can be also observed.
Maize and yellow nutsedge were sown in different rate. The mutual effects on their growing patterns in the first 4 weeks were observed. Since we watered the plants, competition for the light and nutrient could be observed. We also tested the competition between the shoot and roots.
A pot trial in four replications was conducted in a glasshouse, June 2006. The pots were filled with 1 kg sandy loam, with a humus-content of 1,5 %, collected from a field of Sávoly. In each of the pots 5 seeds or tuberlets were sown and planted in different combinations (Table 1). Since the germination rate of maize was 95 %, one more seed were sown in every pot, and later on thinned to the planned plant number. The tuberlets of the yellow nutsedge were presprouted, because the sprouting rate of the tubers was not known. The tuberlets of yellow nutsedge were collected from a field of Somogytúr, cleaned, and presprouted in petridishes on wet filter paper before planting. The plants were grown for four weeks in the pots, every day watered, as they needed, and once a week according to their weight. After four weeks, when maize plants were at 5-6 leaves stage, the plants were harvested, leaf number, the shoot length, root length, fresh weight and dry weight of the plant shoot, and separately also of the roots were measured.

The maize cultivar was a hybrid KWS 328, a new cultivar, ripening when the plant is still green. It has a good stress tolerance and a good resistance to lodging. Date of release: 2003.

Table 1. The treatments and their designations were as follows (these designations are used also in the diagrams)

Treatments (plant numbers in the plots)	Designations
5 maize (control)	5M
5 yellow nutsedge (control)	5Y
4 maize and 1 yellow nutsedge	4M+1Y
3 maize and 2 yellow nutsedge	3M+2Y
2 maize and 3 yellow nutsedge	2M+3Y
1 maize and 4 yellow nutsedge	1M+4Y

Results

Shoot length

Range of shoot length of maize was between 67 cm and 87 cm. Values of the treatments were significantly higher than that of the check plants (5 maize). Range of shoot length of yellow nutsedge was between 28 cm and 32 cm. Values were similar to that of the check plants (5 yellow nutsedge), there were no significant differences. The maize plants reacted with a higher shoot length to the increasing number of yellow nutsedge, suggesting the strong competition for light (Figure 1).

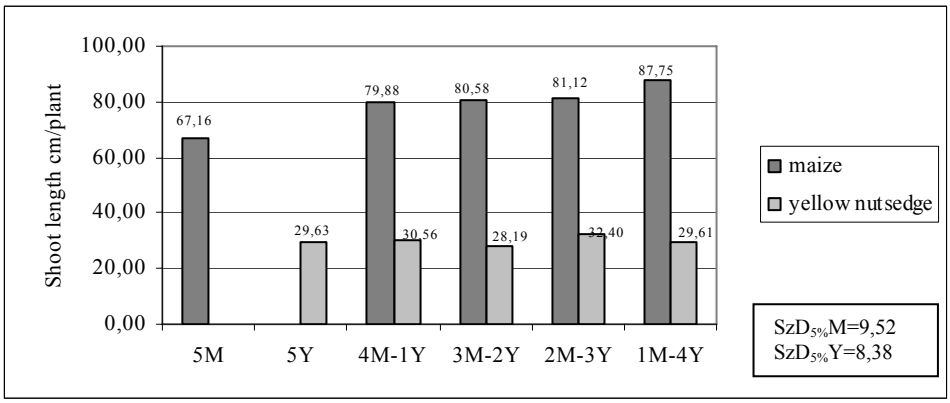

Figure1. Changes of sprout lengths as an effect of plant combinations

Leaf numbers

The leaf number of the maize plants at harvest of the trial was 5-6. Leaf number of the treatment 4maize+1yellow nutsedge and 3maize+2yellow nutsedge was lower than that of the control plants (5 maize), while in the

treatment 2maize+3yellow nutsedge and 1maize+4yellow nutsedge it was higher, in the latter case it was significantly higher. Average leaf number of yellow nutsedge was also about 5, treatments caused no significant difference from the controls (5 yellow nutsedge). Higher values could be observed only at the treatment of 2maize+3yellow nutsedge. Leaf numbers of yellow nutsedge in the treatments 4maize+1yellow nutsedge, 3maize+2yellow nutsedge and 2maize+3yellow nutsedge were higher than that of maize, but in the treatment 1maize+4yellow nutsedge leaf number of maize was higher (Figure 2).

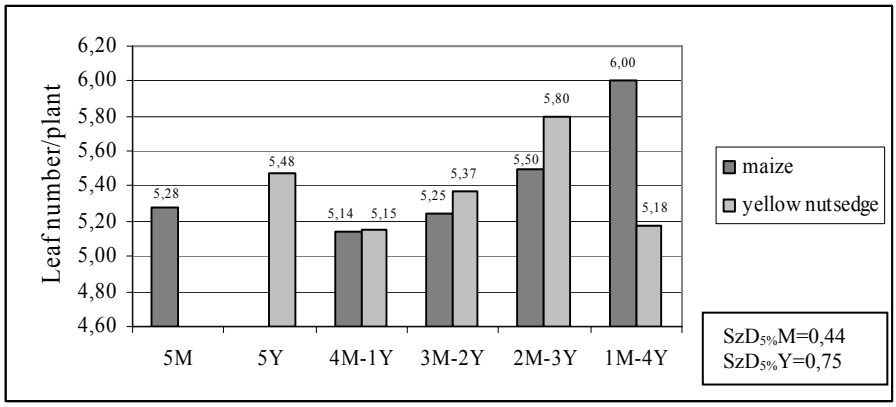

Figure 2. Changes of leaf numbers as an effect of plant combinations

Fresh weight of shoots

The fresh weight of maize shoots changed between 7,91 g and 16,25 g. As a consequence of longer shoot with more leaves, the fresh weight of all maize plants of the combinations exceeded the fresh weight of control maize plants (5 maize). This increase however only at the treatment 1maize+4yellow nutsedge was significant. The fresh weight of yellow nutsedge – similar to the shoot length – was lower than that of the maize plants: ranged between 0,85 g and 1,23 g per plant. Only in the treatment 2maize+3yellow nutsedge was the fresh weight higher than that of control (5 yellow nutsedge) plants. Here there was no significant difference (Figure 3).

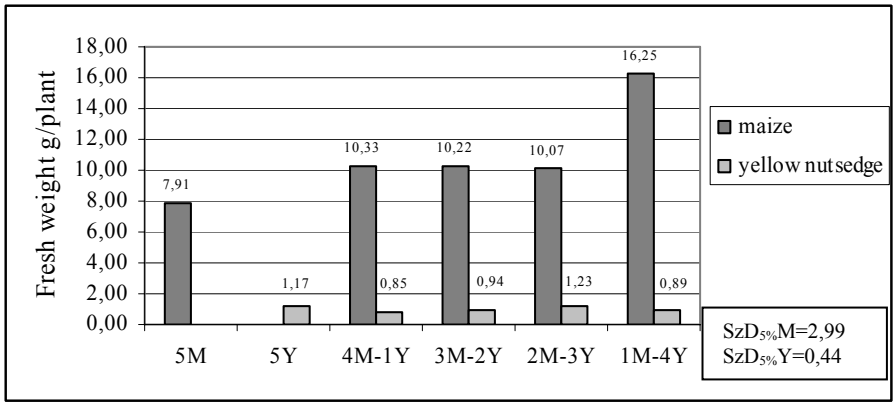

Figure 3. Changes in fresh weights of the shoot as an effect of plant combinations

Dry weights of shoots

The dry weight of the shoots followed the range of the fresh weights. The dry weight of maize shoot changed between 1,06 g and 2,55 g per plant, where the increase in the case of 1maize+4yellow nutsedge was significant. The dry weight of yellow nutsedge shoot ranged between 0,12 g and 0,17 g per plant. The treatments of 4maize+1yellow nutsedge and 2maize+3yellow nutsedge were nearest to the controls (5 yellow nutsedge) in this respect. (*Figure4*).

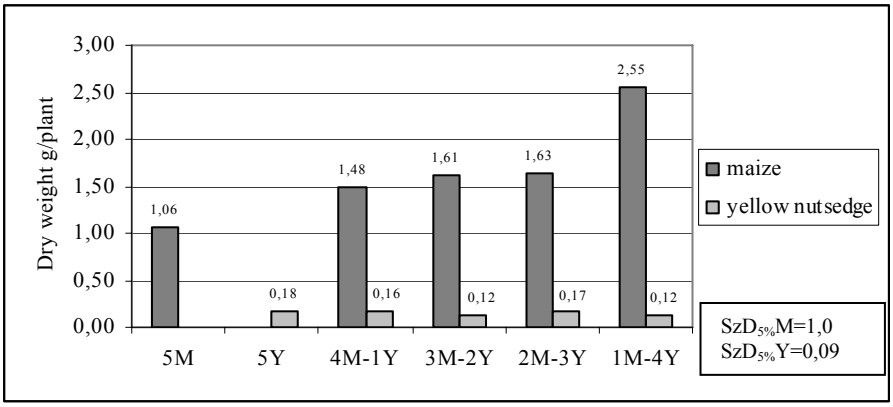

Figure 4. Changes in dry weights of the shoot as an effect of plant combinations

Root length

The root length of maize was between 26 cm and 34 cm. The root lengths in the treatments exceeded that of controls (5 maize), these values were however not significant. Root length of yellow nutsedge ranged between 16 and 27 cm, those of the treatments were lower than those of control plants (5 yellow nutsedge). Root length of yellow nutsedge exceeded that of maize only in case of the controls (Figure 5).

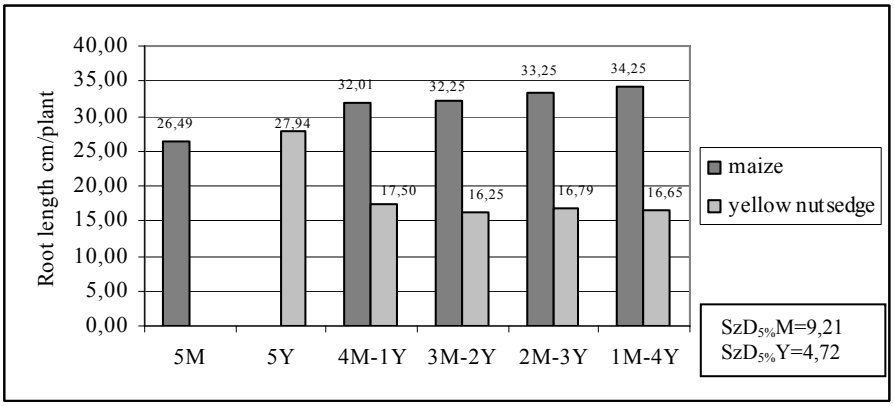

Figure 5. Changes of root lengths in the trials

Root fresh weights

The root fresh weights of maize in the trial changed between 2,87 and 5,28 g/plant. Lower value than that of the control plants (5 maize) was in the treatment 3maize+2yellow nutsedge, while treatments 4maize+1yellow nutsedge, 2maize+3yellow nutsedge and 1maize+4yellow nutsedge exceeded the control value, in the case of the last one it was significantly higher. Root fresh weights of yellow nutsedge ranged between 0,17 g and 0,65 g/plant. In one treatment, 2maize+3yellow nutsedge this value was higher than that of the control (5 yellow nutsedge), but it was not significantly higher. In the case of other treatments the values were lower, and in the case of 3maize+2yellow nutsedge significantly lower. The fresh weights of yellow nutsedge roots were much lower than that of the maize plants (Figure 6).

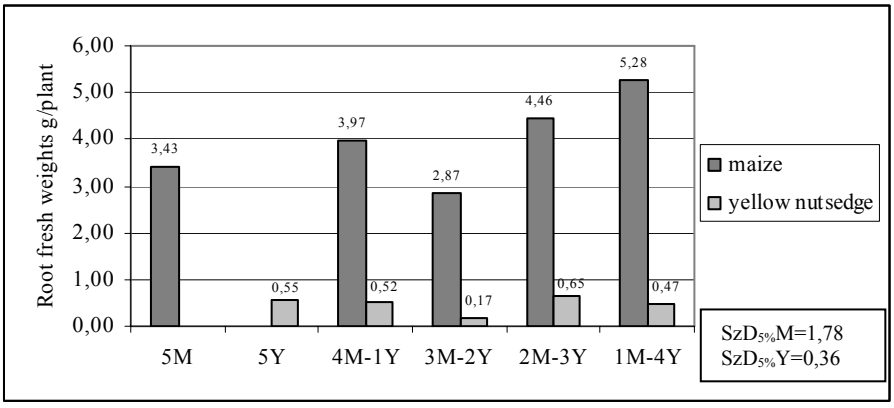

Figure 6. Changes of root fresh weights as an effect of plant combinations

Dry weights of roots

Root dry weights of maize plants ranged between 0,28 and 0,38 g/plant. The treatments 4maize+1yellow nutsedge and 3maize+2yellow nutsedge showed

lower values than that of the controls (5 maize), and 2maize+3yellow nutsedge and 1maize+4yellow nutsedge showed higher values, still all these differences were not significant. Root dry weights of yellow nutsedge ranged between 0,03 and 0,05 g/plant. Similar to root fresh weight, plants of 2maize+3yellow nutsedge treatment showed higher value than controls (5 yellow nutsedge), the other were lower. No differences were significant. Similar to the root fresh weights, the root dry weight values of yellow nutsedge were much lower than that of maize dry root weights (Figure 7).

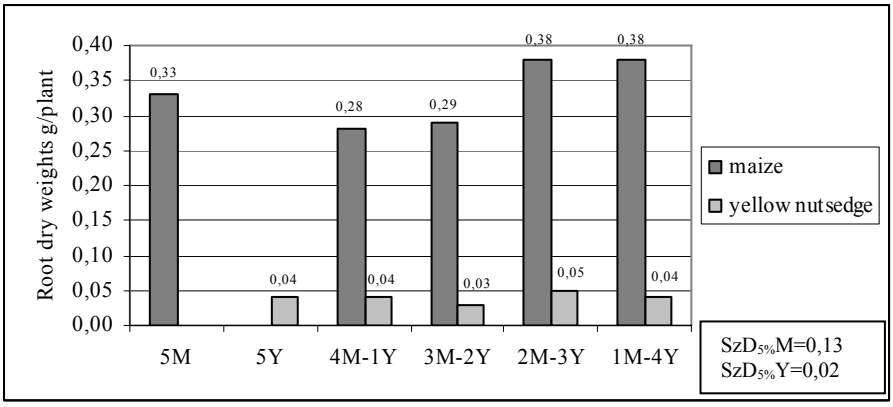

Figure 7. Changes of root dry weights as an effect of plant combinations

Conclusions

It can be stated, that the cultivated plant could successfully compete with the weed. The maize reacted with a vigorous shoot- and root growing to the presence of higher weed density. The growing values of the maize plants in the maize-nutsedge combinations definitely exceeded the growing values of the control plants (5 maize) in all the tested parameters, like shoot length, shoot and root fresh weight, shoot and root dry weight. The values of shoot length, leaf number, shoot fresh and dry weight, and root fresh weight were significantly higher. The intraspecific competition was stronger in the case of the maize plants, since the maize plants showed lower values alone, than in the mixtures.

In the case of the yellow nutsedge the measured values were near to that of the controls (5 yellow nutsedge). Higher values were observed in the treatment 4maize+1yellow nutsedge for shoot length, and in the treatment 2maize+3yellow nutsedge for shoot length, leaf number, shoot fresh weight, fresh and dry root weight. The root length and root fresh weight of the nutsedge significantly decreased in the combinations. In the case of the yellow nutsedge the interspecific competition proved to be stronger.

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INVESTIGATION ON THE EARLY COMPETITION BETWEEN YELLOW NUTSEDGE (*CYPERUS ESCULENTUS* L.) AND MAIZE

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Summary

The yellow nutsedge is a tuber bearing perennial weed plant. It spreads quickly by its huge amount of seeds and tuberlets, and may present a big concurrence to the cultivated plants, causing yield and quality damages. It occurs mainly in root crops.
We investigated, that maize and yellow nutsedge sown in different rate, what like mutual effect have on the growing patterns in the first 4 weeks. A pot trial in four replications was conducted in a glasshouse. In each of the pots 5 seeds or tuberlets were sown and planted in different combinations. Four weeks later we counted the leaf number, and measured the shoot length, root length, fresh weight and dry weight of the plant shoot, and separately also of the roots.
Summarized it can be stated, that the cultivated plant could succesfully compete with the weed. The maize reacted with a vigorous shoot- and root growing to the presence of higher weed density. The growing values of the maize plants in the maize-nutsedge combinations definitely exceeded the growing values of the control plants (5 maize) in all the tested parameters, like shoot length, shoot and root fresh weight, shoot and root dry weight. The intraspecific competition was stronger in the case of the maize plants, since the maize plants showed lower values in clean crop, than in the mixed treatments.
In the case of the yellow nutsedge the measured values were near to that of the controls (5 yellow nutsedge). Only the root length and root fresh weight of the nutsedge were significantly lower in the combinations. In the case of the yellow nutsedge the interspecific competition proved to be stronger.

AMBROSIA ARTEMISIIFOLIA AND *IVA XANTHIFOLIA* SPREAD AND DISTRIBUTION IN VOJVODINA REGION

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In the last four years, due to the high influence of Ragweed pollen as allergen to human health and working capability in the region of the City of Novi Sad a project of its control was established. During project accomplishment, *Iva xanthifolia* Nutt. presence and spread was also confirmed. Therefore, along with Ragweed control in the phase before flowering and it's mapping, in the frame of the project, the same method was established for allergenic *Iva xanthifolia* Nutt.

Literature

Ambrosia artemisiifola L. (Family *Asteraceae*), commonly called "Ragweed" is weedy ruderal species of fast and intensive spreading. It has been transferred from neighboring European countries into ours (Konstantinovic, 1999). It belongs to adventive floristic elements, and it was introduced from North and Middle America into Europe in 1800 together with clover seed (Priszter, 1960? Hansen, 1976). In USA and Canada it is considered to be one of the most significant weeds and its eradication is regulated by numerous acts of low. While spreading, Ragweed began to adapt also to open vegetation on non-agricultural land, mostly of ruderal type, on degradated meadows. It can be found in almost all crops and plantations. It is fast spreading and becomes cosmopolitan. Due to the wide ecological valence it easily becomes dominant weed in different conditions. In it's life form it is terophyte, which means that unfavourable life period survives in the form of seed (Soó, 1970). It is thermophile species, sprouting through all spring and summer. Emergence begins with favourable soil temperatures and lasts until harvest. However, on ruderal sites it emerges even until September. It fructifies abundantly with 150 000 seeds per plant annually, thus making its control extremely difficult and expensive. Ragweed seed can maintain germination capacity in soil for over 40 years, which indicates extremely high reproductive potential and permanent seed bank in soil (Levente et al., 2003).

Iva xanthifolia Nutt. (Family *Asteraceae*), Marsh elder, False ragweed is annual, termophyl native Canadian weed (Scoggan, 1978).

Marsh elder is a robust plant of over 2m in height , highly competitive with abundant seed production of more than 80.000 seeds/plant. During 2nd half of 19th century Marsh elder began to spread in Europe (Hejny, 1958). In 1966 the first report of Marsh elder presence in four localities of Vojvodina, near Novi Sad was issued. During 70th years of the last century, it continued spreding and its presence was recorded in 21 localities, predominantly along roads and tracks (Sajinovic and Koljadzinski, 1978). Since 1990 the first reports on Marsh elder presence were also given for arable crops. (Veljkovic, 1996.). Marsh elder already causes problems in agricultural crops, specially in row ones.

Materials and Methods

During vegetation period 2004 and 2005, on areas under horticultural plants a combined evaluation of Ragweed and Marsh elder number and coverage was accomplished by Braun-Blanquet method (1951).The most efficient and the lowest toxical measures of chemical control of this allergentic species were established along with use of efficient low toxicity herbicides that are sanitary and environmentally sound, as well as mechanical control measures by mowing.

Results

Ragweed has been found on both banks of the river Danube, near the suburbs of Petrovaradin, Sremski Karlovci and the city of Novi Sad, as well as in Bogojevo, Odzaci, Bac, Backa Palanka. The weed has also been spreading northwards towards Kula, Begec and Futog of the Backa Region and toward central and south Banat Region. During 2004.godine the studies were performed in the City of Novi Sad, the capital of Vojvodina (Table 1) on over 90 ha of non-agricultural land. Ragweed has been found on over 100 locations in 21 city zones. Invasive species *Iva xanthifolia* Nutt. was determined at one locality in the city Zone Salajka with 6 individuals per m^2.

During 2005 about 400 ha of non-agricultural land was studied in surrounding suburbs of Novi Sad in which Ragweed was recorded in over 200 locations (Table 2), and *Iva xanthifolia* Nutt. in 23 locations.

Table 1. Ambrosia artemisiifolia L. number and coverage in the city of Novi Sad

City zone	2004
Liman	2.2
Detelinara	4.4
Donji Ribnjak	1.1
Kej	2.1
Novo Naselje	2.3
Mali Beograd	1.1
Avijatičarsko naselje	4.4
Industrijska zona	4.4
Sremska Kamenica	2.3
Petrovaradin	3.4
Dunavac-Ribarsko ostrvo	4.4
Stari grad	1.1
Gradsko groblje	3.2
Institut za topolarstvo-Kaćka šuma	3.3
Highway Novi Sad-Beograd	5.5
Veternik	4.4
Salajka	3.2
Miševluk	3.4
Telep	3.3
Kamenjar	2.2
Adice	1.1

Table 2. *Ambrosia artemisiifolia* L. number and coverage in suburbs of the city of Novi Sad

City zone	2005
Pejićevi Salaši	4.2
Čenej	5.3
Futog	4.3
Veternik	4.3
Begeč	5.3
Kisač	4.3
Stepanovićevo	4.3
Rumenka	4.3
Bukovac	1.1
Petrovaradin	3.2
Budisava	2.3
Kovilj	3.4
Šangaj	4.4
Kaćka šuma-Kaćki atar	4.3
Kać	3.4
Rafinerija	4.4
Novo Naselje	3.3
Kamenjar	2.3
Industrijska zona	3.3
Nemanovci	3.3

In Table 3 number and coverage of Rugweed at the territory in suburbs of the city of Novi Sad during 2006 is presented. *Iva xanthifolia* presence was recorded at 32 locations.

Table 3. *Ambrosia artemisiifolia* L. number and coverage in suburbs of the city of Novi Sad

City zone	2006
Rumenka	3.3
Kisac	3.3
Stepanovicevo	3.3
Cenej	4.4
Sangaj	4.4
Begec	3.1
Pejicevi Salasi	3.2
Crossroad with a highway, approach to Novi Sad	3.4
Kovilj	5.4
Highway / City disposal area	5.2

In last two years systematic monitoring of Rugweed occurrence has been perfomed for the territory of the Community of Zrenjanin in which in 2005 this species was determined and control in the area of 93 090 m^2, and in 2006 in the area of 93 750 m^2.

Discussion

Weedy ruderal species *Ambrosia artemisiifolia* L., is wide spread in whole Vojvodina. It often builds huge, compact communities mostly in ruderal sites. In cooperation with the Administration of the City of Novi Sad, Department for Municipal Business, in the frame of the project, mapping, determination of number and coverage, as well as control of the allergenic species *Ambrosia artemisiifolia* L. and *Iva xanthifolia* Nutt. in the city of Novi Sad and it's suburbs. Occurrence of new generations, i.e., monitoring of this species, as well as retro vegetation which is more frequent after mowing on rough terrains were permanently recorded. During vegetation period, these allergenic species have been controlled by repeated mowing, while in ruderal sites inconvenient for mowing such as road and railway sides and non-agricultural land in suburbs were sprayed by herbicides based on glyphosate in the quantity of 4 l ha^{-1}. Two years of *A. artemisiifolia* L. monitoring and control resulted in significant reduction in

its number. As Ragweed and Marsh elder are weedy ruderal species that also spread in the nearest surrounding of the city on agricultural land under crops, it is necessary to suppress it to fructification. Recommended cultural practices for its control include harrowing in the phase of emergence, keeping crops free of weeds and mowing in non-agricultural land. Depending upon crop variety various herbicides may also be applied. In regard to previous year, in 2005 monitoring of Ragweed pollen quantity in the air showed reduced pollen quantity per $1m^3$. The average number of pollen grains was 143 per m^3, while in 2003 it was 657 grains per m^3. In some localities presence of newly introduced ruderal species in our country *Iva xanthifolia* Nutt., was also been determined and monitored. Danger of this species is perceived in strong concurence, huge seed production, and in immense allergic features.

Application of combined mechanical and chemical control measures in the area of the city of Novi Sad and it's suburbs led to reduction of *Ambrosia artemisiifolia* L.and *Iva xanthifolia* Nutt. species. However, problem of this invasive species spreading is not permanently solved, for remaining non-agricultural and agricultural land in Vojvodina represents constant seed bank that endangers the city and its surronding. Due to this, *Ambrosia artemisiifolia* L. and *Iva xanthifolia* Nutt. are necessary to be controled in soybean, sugarbeet, maize and sunflower crops by application of contact herbicides. On non-agricultural land they should be controled by mechanical measures such as mowing or by chemical control.

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AMBROSIA ARTEMISIIFOLIA AND *IVA XANTHIFOLIA* SPREAD AND DISTRIBUTION IN VOJVODINA REGION

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Summary

During last years, relatively reduced crop rotation, shallow tillage, inadequate pre-sowing cultivation and use of the same or identical herbicide groups enabled spread and domination of the weed species *Ambrosia artemissifolia* L., *Iva xanthifolia* Nutt. and *Xanthium strumarium* L. In last years *Iva xanthifolia* Nutt., newly introduced weed species in our country, represents great problems especially in row crops. From the aspect of the human health this species is very dangerous, for it is, like the species *Ambrosia arthemisiifolia* L. particularly allergenic. It is naturalized in central-east and south-east Europe and France, and in other regions it is of sporadic occurrence. In 1800, *Ambrosia artemisiifolia* L., ruderal weed species of fast and intensive spread that belongs to adventive floristic element was introduced to Europe from North and Middle America. While spreading, it began to adapt to open vegetation on mostly of ruderal or weed type terrains. Now it invades natural vegetation of semi-closed type, such as degraded meadows. It weeds almost all crops and plantations it is fast spreading and becomes cosmopolitan. In our country it has been determined in greater number of localities.

STUDY OF WEED SPECIES *ECHINOCHLOA CRUS-GALLI* L. CROSS-RESISTANCE

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Herbicide resistance is acquired plant ability to survive herbicide rate that is normally lethal for wild biotypes and to continue reproduction. The most important factor that causes evolution of herbicide resistant weed species is selection pressure of the applied herbicide (Jasieniuk *et al*., 1996). This pressure is increased by use of higher herbicide rates, high efficiency and/or persistency and in cases in which there is no rotation of herbicides with different mode of action (Shaner, 1995). Expansion of use of herbicides based on ALS inhibitors caused resistance development and new problem in weed control. Until 1993 number of confirmed ALS resistant cases was 14, and in 1999 it numbered even 58 cases (Heap, 1999). Today in the world 310 resistant biotypes of 183 weed species of which even almost 95 are ALS resistant ones have been determined. (HRAC, 2006). Cross resistance, incidence of which lately has become more frequent represents resistance to more herbicides of the identical action mechanism. Extremely high number of cases has been determined for chemical family of sulfonylureas.

Results of biological studies of weed resistance to herbicide exclude use of inefficient herbicides, which reduces expenses in agricultural production and prevents spread of resistant biotypes.

Literature

Since the beginning of ALS inhibitors use in 1982, they represent one of the most significant herbicide classes. Low quantity of use, wide spectrum of weeds that they control, flexible application time and low toxicity contributed to fast acceptance of these herbicides. Today there are even five, structurally different chemical classes of ALS inhibitors: imidazolinones, sulfonylureas, triazolopyrimidines, pyrimidinylthio-benzoates and sulfonylaminocarbonyltriazolinones (Tranes and Wright, 2002) of which imidazolinones and sufonylureas are of the widest use. Primary action site of these herbicides is the enzyme of acetolactate

synthesis that is responsible for synthesis of amino acids valine, leucine and isoleucine (Babczinski, 2002). Due to their continuous use in subsequent years, the greatest disadvantage of this herbicide group is extremely fast development of resistant biotypes (Duran-Prado et al., 2004).

The primary action site of cross-resistance is between sufonylureas and triazolopirimidines is mutation at $Proline_{197}$ (Saari et al., 1990). However, primary action site of cross-resistance effect between imidazolinones and sufonylureas is more difficult to find out, for it is based on multiple mutation sites (Foes et al., 1999). Occurrence of cross-resistance to ALS inhibitors in weed species *Echinochloa crus-galli* L. has not yet been determined (HRAC, 2006); while in 1988 cross-resistance was established for biotype *Datura innoxia* to imidazolinones and sufonylureas. (Saxena and King, 1988).

The aim of these studies was to identify resistance of *Echinochloa crus-galli* L. biotypes from different localities in Vojvodina to imidazolinones and assessment of the existing cross-resistance to sufonylureas.

Materials and Methods

For resistance studies to herbicides, seed of *Echinochloa crus-galli* L. was collected from various localities in Vojvodina (localities A, B and C). Susceptible referent population was collected from untreated sites (SS – susceptible standard). Resistance study to imidazolinones (herbicide imazethapyr) and sufonylureas (herbicide nicosufluron) was performed by application of two methods, Petri dish bioassays (Clay and Underwood, 1990) and by whole plant studies (Moss, 1995). Herbicides were applied in the range of rates: 0.04; 0.08; 0.10; 0.15; 0.20; and 0.40 kg a.i imazethapyr/l and 40; 50; 80; 120; 160 and 240 g a.i. nicosufuron/l; control remained untreated. Resistance level determination was performed upon resistance index (IR) which is calculated according to the following formula:

$IR = ED_{50}R / ED_{50}SS$
R – Resistant population
SS – susceptible population

where ED_{50} is for the rate that causes reduction of the measured parameter for 50% in regard to untreated control (Moss et al., 1998). IRs' are presented for all morphological parameters, such as epicotyls and hypocotyls shoots length, stem height, foliage fresh weight. Resistance level was determined also upon reduction of foliage fresh weight by scale according to Moss et al. (1999) (Table 1).

Table 1. Scale of susceptibility level (Moss et al., 1999)

susceptibility level	
S	susceptibility to the applied herbicide
1*	early indication of resistance, possibility of reduction in herbicide action
2*/3*	confirmed resistance, possibility of reduction in herbicide action
4*/5*	confirmed resistance, slight possibility of poor herbicide action

Statistical processing of the measured parameters values was performed by variance analysis (ANOVA) and significance difference was evaluated by t-test (Hadzivukovic, 1991). Results of the measured parameters are presented graphically and in tables.

Results

In Figures 1 and 2 shoots epicotyl's lengths of *Echinochloa crus-galli* L. are given at series of imazethapyr and nicosufluron rates, whereas on Figures 3 and 4 are presented shoots hypocotyls lengths from the studied localities.

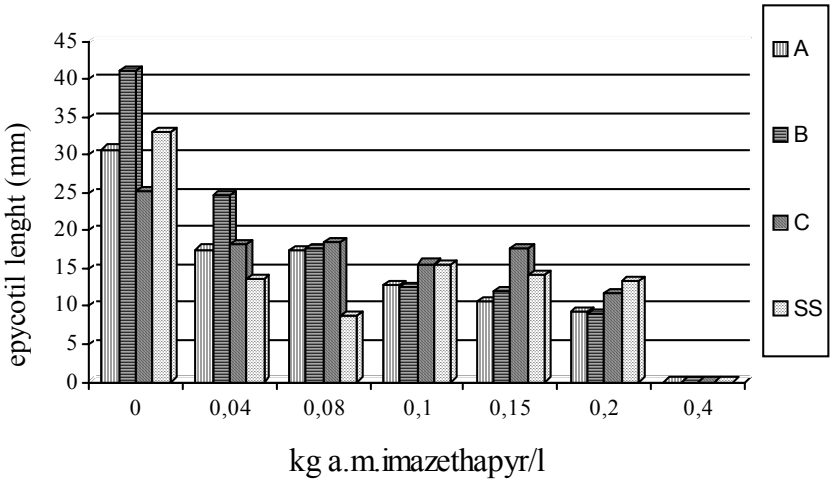

Figure 1. Epicotyl's length of *Echinochloa crus-galli* L. shoots given at series imazethapyr rates

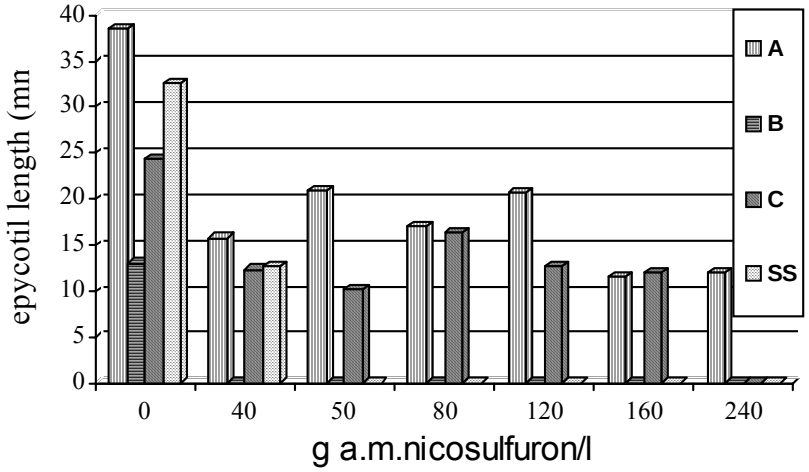

Figure 2. Epicotyl's length of *Echinochloa crus-galli* L. shoots given at series nicosufluron rates

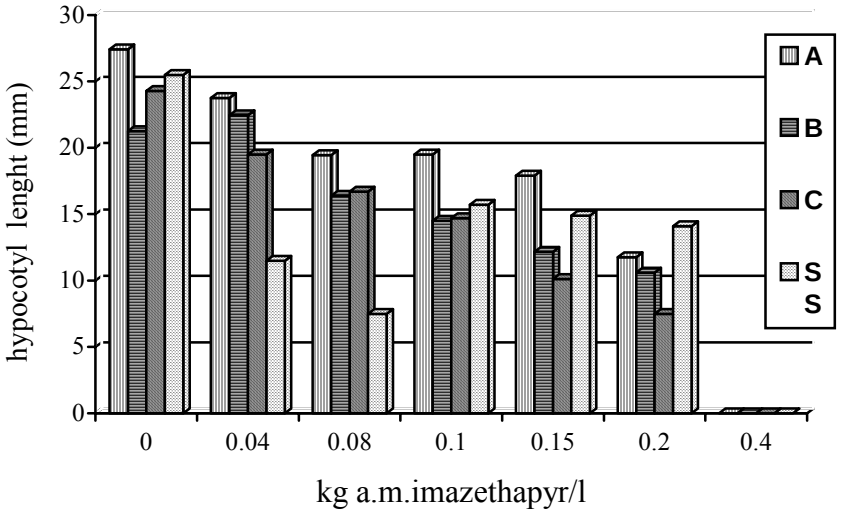

Figure 3. Hypocotyls length of *Echinochloa crus-galli* L. shoots given at series imazethapyr rates

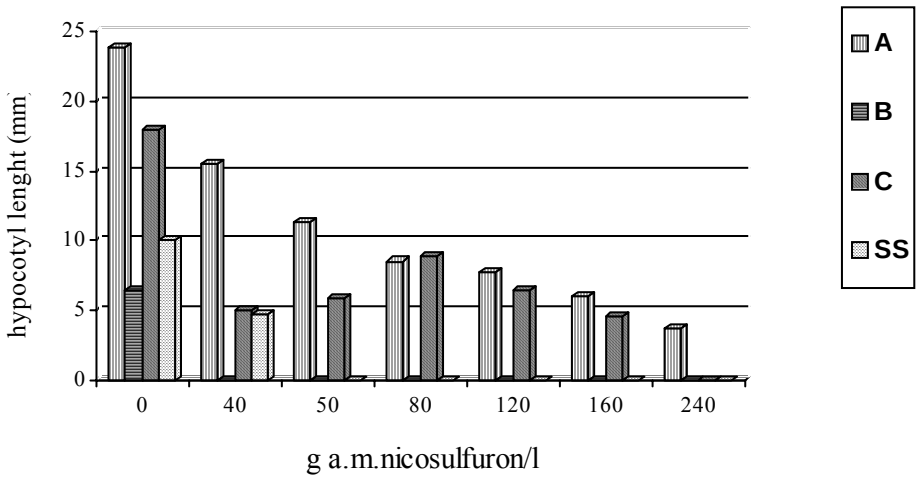

Figure 4. Hypocotyls length of *Echinochloa crus-galli* L. shoots given at series nicosufluron rates

Stem height of *Echinochloa crus-galli* L. plants was measured at various rates of the applied herbicides (Figures 5 and 6).

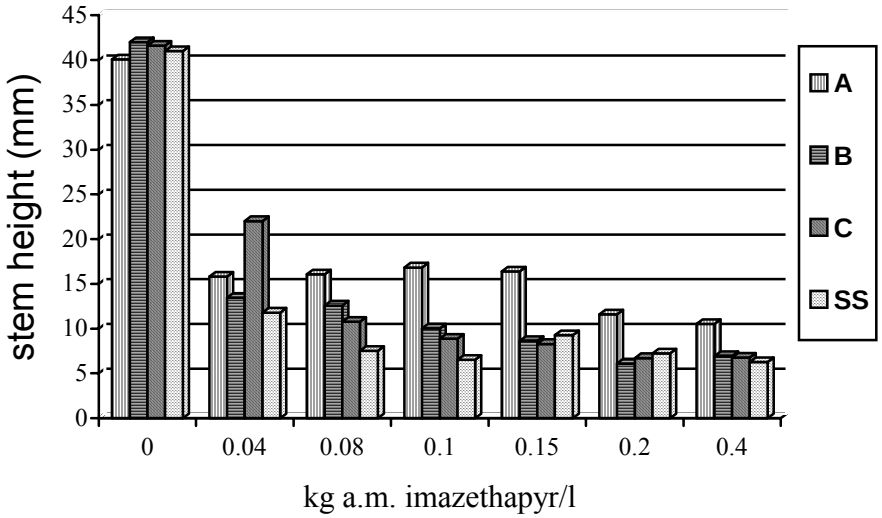

Figure 5. Stem height of *Echinochloa crus-galli* L. plants at series imazethapyr rates

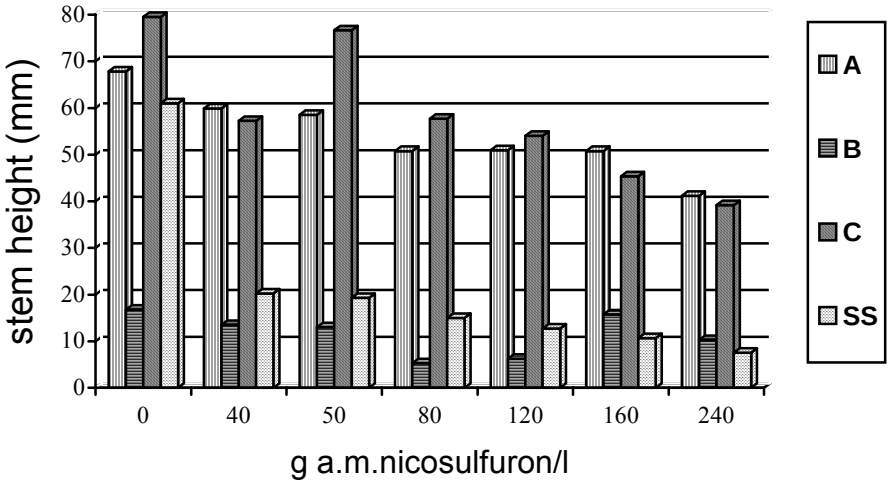

Figure 6. Stem height of *Echinochloa crus-galli* L. plants at series nicosufluron rates

Resistance level based upon fresh foliage weight was measured according to scale by Moss et al. (1999) that implies several possible resistance levels of the studied population - 1*, 2*, 3*, 4* and 5* (Table 2).

Table 2. Resistance level of the studied *Echinochloa crus-galli* L. biotypes according to the scale by Moss

Biotype	Resistance level imazethapyr	Resistance level nicosufluron
A	4*	5*
B	4*	2*
C	3*	4*

Resistance of *Echinochloa crus-galli* L. biotypes to herbicides imazethapyr and nicosufluron is presented by resistance index (Tables 3 and 4).

Table 3. Resistance index to herbicide imazethapyr of the studied *Echinochloa crus-galli* L. biotypes

Biotype	I R			
	Epicotyl	Hypocotyl	Stem height	Foliage fresh weight
A	1.72	1.90	1.38	1.42
B	1.27	1.65	1.27	1.33
C	1.05	1.5	1.19	1.25

Table 4. Resistance index to herbicide nicosufluron of the studied *Echinochloa crus-galli* L. biotypes

Biotype	I R			
	Epicotyl	Hypocotyl	Stem height	Foliage fresh weight
A	∞	∞	1.31	1.59
B	0.0	0.0	0.80	0.62
C	∞	∞	1.05	1.41

Statistically significant differences ($p<0,05$) between biotypes from all studied localities and biotype used as susceptible standard were determined by analysis of imazethapyr effect to morphological parameters of the species *Echinochloa crus-galli* L. (t test), and values of the studied parameters from localities A and C were perfomed by analysis of nicosulfuron effect to morphological parameters.

The lowest percentage of germinated seed was established for biotype *Echinochloa crus-galli* L. from locality B, after treatment by herbicides imazethapyr and nicosulfuron (Figures 1 and 2). Biotypes from localities A and C germination capability after both of the applied herbicides had values 75-100%. Good development of shoots after imazethapir treatment was determined for all biotypes from all studied localities, while after nicosulfuron treatment biotypes from localities A and C were well developed and had the highest values for epycotil and hypocotyl lenghts.

Significantly heigher persentage of emerged plants of biotypes from localities A and C was determined after nicosulfuron treatment, while emergence of plant biotypes from locality B afther both of the applied herbicides had low values. Resistance to the herbicide imazethapyr of biotype *Echinochloa crus-galli* L. from the studied localities was confirmed by values for stem height, while nicosulfuron resistance was d determined by measurement of the same parameter for biotypes from localities A and C.

There was no decay of plants from these localities even at nicosulfuron rates of 240 g a.m. nicosulfuron/l, which is six times heigher from usually applied rates.

Based upon values given in Table 2, resistance to imazethapyr was confirmed for biotypes from localities A and B (4*), with a slight probability that herbicide exibited poor action. Resistance for biotype from locality C was confirmed with a probability of herbicide action reduction. Nicosulfuron resistance, based upon fresh foliage weight was confrimed for biotypes from localities A (5*) and C (4*). Resistance of biotype from the locality B was confrimed, with a possibility of herbicide action reduction.

Based upon IR of all measured parameters (Table 3), the highest resistance to the applied imazethapyr rates was determined for biotype *Echinochloa crus-galli* L. from the locality A (1.38-1.90), while somewhat lower IR values were determined for biotype from the locality B (1.27-1.67) and C (1.05-1.25). Such IR values indicate resistance evolution to imazethapyr of biotypes from all of the studied localities.

For biotype from the locality B (Table 4), susceptibility to the applied nicosulfuron rates (IR=0-0.80) was determined. IR values for stem height and fresh foliage weight of biotypes from localities A (1.31;1.59) and C (1.05; 1.41) indicate outbreak of resistance. According to IR of these biotypes, determined upon epycotil and hypocotyl lenghts, resistance to the herbicide nicosulfuron was established.

By the comparative analysis of the presented results, outbreak of resistance development to imazethapyr (imidazolinones) was determined for biotypes of the weed species *Echinochloa crus-galli* L. from localities A, B and C, and to the herbicide nicosulfuron (sulfonilureas) for biotypes from localities A and C. Biotype of Echinocholoa crus-galli L. from locality B remaind susceptible to the herbicide nicosulfuron.

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STUDY OF WEED SPECIES *ECHINOCHLOA CRUS-GALLI* L. CROSS-RESISTANCE

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Summary

Long lasting use of the herbicide or herbicides with the identical action mechanisms causes selection of resistant and elimination of the susceptible biotypes of different weed species. During 2005 and 2006 weed species *Echinocloa crus-galli* L. resistance was studied to ALS inhibitors. Seed of the studied weed species were collected from different sites of Vojvodina with a long history of ALS inhibitor's use. Population from ruderal site was used as a susceptible standard. Study was performed according to Clay and Underwood (1990) and Moss (1995) method. Resistance level was determined by resistance index for morphological parameters such as epicotyls and hypocotyls length, stem height, foliage fresh weight.

Initial development of imazethapyr resistance (imidazolinones) was determined for biotypes of the weed species *Echinochloa crus-galli* L. from locality Kamendin (A), Bac (B) and Kozarica (C) by comparative analysis of the obtained data. Biotype of *Echinochloa crus-galli* L. from locality B remained susceptible to the herbicide nicosufluron.

POSSIBILITIES OF INTEGRATED WEED CONTROL AGAINST JOHNSON-GRASS (*SORGHUM HALEPENSE* /L./ PERS.)

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The Johnson-grass (*Sorghum halepense* /L./ Pers.) is a perennial monocotyledon. Its gen centre is in the Near East. Botanically this weed belongs to the monocotyledone class, *Poaceae* (*Gramineae*) family and within this, together with the maize to the *Andropogonoideaei* subfamily (Simon, 2000).

Pál Kitaibel mentioned its occurrence as early as 1800. Though it was present in the first decades of the 20 century, it was not an important weed. It was living mainly on ruderal areas, and on the arable fields only thread by thread. Its importance later on represented by its order in the weed survey lists made in the previous century. At the time of the First National Weed Survey (1947 – 1953) it has not been found in Hungary. In the periods of the 2nd (1969 – 1971), 3rd (1987 – 1988) and 4th Survey it occupied the 94th, 18th and 10th place of importance, respectively (Tóth and Spilák, 1998). Because of its rapid multiplication and spreading after the second weed survey (1969-1971) and because of its very complicated and difficult control, the Plant Protection Division of our Ministry of Agriculture ranged it in 1974 into the category of „Dangerous weed" (Hunyadi et al., 1994).

Damages by this weed was observable from the first part of the 20th century on. Still, Miklós Újvárosi, a weed scientist did not think the weed will propagate in Hungary. He supposed, the cold winters and hard frosts will hinder its spreading. Újvárosi (1973) as written in his book „Weeds, weed control" (Gyomnövények, gyomirtás) considered it to be only a ruderal weed, and mentioned its frost sensitivity as a natural hindrance of its distribution. Results of later biological findings however disproved this meaning. But the book edited by Hunyady (Szántóföldi gyomnövények és biológiájuk, 1988) on weeds and their biology on arable fields, states, that „according to present research results frost tolerance of the overwintering rhizomes is good". This statement has been proved later on by the rapid spreading of Johnson-grass from the middle of the 1960s.

The big farm sizes played a role in the spreading of this weed, since the tilling and harvest machines spreaded its seeds on the large fields during a relatively short period. As another important factor can be mentioned

onesided using of atrazin, which do not kill Johnson-grass, but suppresses broadleaves weeds, which could prevent development of Johnson-grass by shading and occupation of the place. Deeper pluggings also helped the overwintering of the root-stocks (Hunyadi et al., 2005). Up to the present it became obvious, that if this weed settled, practically it is impossible to destroy its rooot and branches. Using present herbicides one can restraint the damages on the infected fields, or keep it on an economically acceptable level. Still, we have to live together with it.

According to experts the main causes of its fast distribution in Hungary are:

- the relatively mild winters of the last decades,
- introduction of its seeds by seeds and machines,
- the relatively deep ploughings in the big farms, cooperatives,
- frequent using of atrazine,
- the allelopatical character of the plant,
- its apical dominance,
- since it can propagate themselves both from seeds and rhizomes, the double protection against it can not be made easily.

The protection against this weed is significally hindered by the fact, that in his propagation both rhizomes and seeds play nearly the same importance. The life cycles of the plants are very similar in both types of propagation, though on the fields the propagation by rhizomes mostly precedes that of from seeds, since the growing of sprouts is faster from the rhizomes. Three weeks after emergence starts tillering and growing of lateral shoots. The intensive rhizome formation takes place during the flowering, lasting from the 7th week of the life cycle up to the end of the vegetation (Hunyadi et al., 1994). A plant may produce cca 80.000 seeds. Its seed yield may be as much as 900 kg/ha (Mc Whorter, 1973). The seeds have a primary dormancy. The seed viability in soil lasts for 3 – 6 years. The plants emerging from seeds start to initiate rhizomes just three weeks later, it means that only a short time they need to achieve their perennial character (Kovács, 2002). The spreading of this weed takes place mainly by seeds. The stability and agressivity of the plant following its settlement will be ensured by its rhizomes growing in the 5 – 20 cm depth of the cultivated soil layer. It has an unbelievable ability to propagate, since the amount of rhizome may be 7 – 9 metric tons per hectares (Szabó, 1972). Growing of rhizomes is intensive during the flowering. 90 % of the finger-thick rhizome mass are placed in the upper 15 – 20 cm soil layer (Hunyady et al., 1979).

The Johnson-grass decreases yields of the cultivated plants mainly through the direct concurrence (Takács, 1973), and at the same time it may be host of virus diseases, like maize dwarf virus /MCDV/, (Thorneberry, 1966), sugarcane mosaik virus /SMMV/, (Arceneaux, 1967), potato virus Y /PVY/.

The most effective way of protection is the prevention of its introduction. The only chance to do it is the knowledge and keeping all elements of field hygiene. Among them is important the cleaning of all machines, implements used on an infested field. The introduction by seeds can be avoided by using controlled pure seeds of the cultivated plant. However if it appeared, it has to be killed immediately, since the effectivity of the control in case of seeds is much better, then in case of settled rhyzomes. This weed should be killed by using every possible way. The protection of the cultivated plant against this weed needs an integrated activity, using of all possibilities (Mikulás, 1979).

1. Preventive protection

The prevention is a definitive element of the integrated regulation. Its aim is hindering of spread from uncultivated fields, hindering its introduction by seeds, by harvest wastes, machines, by stable manure, implements.

1.1. Biological protection

Presently it has a small role in the protection, but for a long term by making perfect the methods, using of this method may come into the foreground. For this purpose a species-specific powdery smut were used in form of suspension sprayed onto the stubble. This infected systematically the Johnson-grass, making it dwarf. After a two-years application the Johnson-grass lost its competition ability and agressivity (Quimly, 1982). Mikulás determined applicability of the of the microorganism *Pseudomonas syringae* var. *syringae* on Johnson-grass, and this method has been admitted into the possible biologic al methods. The use this method in the practice, still needs further investigations. Application of allelopathia for plant protection would be possible, if this phenomenon would exist in the wild form of the cultivated plant, and this could be introduced into cultivated forms, modern varieties. It was supposed earlier, that all the cultivated species have had their allelopathy, but this character has been lost during their breeding (Walker, 1982).

1.2. Defoliation

Consecutive defoliations of the Johnson-grass from rhyzome (e.g. with diquat-dibromid) is impracticable, that is why this method can be used succesfully only on emerging plantlets (Mikulás, 1976).

2. *Agrotechnical methods*

2.1. Mechanical protection

This method is not effective for itself, it will complete the other protecting methods. It can be made by mowing on ruderal areas, or by mechanical soil preparation on arable fields and on stubble-fields. The mowing aims

exhausting of nutritive materials stored in the rhyzome. That is why frequent mowing or soil preparation is necessary, since the recreated plant able to replace exhausted nutritions within three weeks after the treatment if regrowed the sprout. By this method the further plant propagation or its mild suppression can be achieved in case of rhyzome originated plants. The mowing effectively prevents the spread by seeds. The soil preparation implements cut rhyzomes into little bits, and by this the axial buds get rid of the hindering effect of the apex, and develop more new sprouts. By this the nutrition consumption of the plants increases, otherwise the leaf area grows in relation to the rhyzome amount, and by this the killing effectivity of the later treatments increases. The plug takes rhyzomes onto the soil surface, they will die because of drying out and by frost. The complete fallowing and using disc-tiller and cultivator in periods of 4 – 5 weeks has a secondary effect onto the decreasing of nutrition and water household of the Johnson-grass, since the disc-tiller cut the rhyzomes into pieces (Verma and Bhardwaj, 1965). The advantage of the cultivator, that it lifts more rhyzome onto the soil surface (Kádár, 1974). The methods of stifling into the soil and exhausting is based on the theory, that disc-tillering in criss-cross will broke rhyzomes into small pieces, which plugged down to 20 cm and ring rollered with a heavy implement, at anaerobic environment will die. The drying out of the rhyzomes is an important part of the control. Shorter rhyzomes in a dry soil dry out easier than longer ones, that is why the frequent treatment is better (Mc Whorter, 1972a). Rhyzomes of Johnson-grass will die when the soil humidity is lower than 30 % of the absolute moisture capacity (Kiselev, 1971). Utilizing of this method depends on the wheather, since dying of the rhyzomes presume a long dry period early spring or following the harvest, and this is occurs seldom in Hungary.

2.2. Crop changes, crop rotation

The *Sorghum halepense* has high demand on light, heat and nutritions, therefore when the cultivated plant grow faster and higher, it will give a shadow, and the Johnson-grass will be suppressed (Újvárosi, 1970). A proper crop rotation will decrease the damages made by moderate grass infection. A crop rotation, which ensures a concurrence against development of the rhyzome, will suppress the rhyzome (Kőrösmezei, 1982). The market posibilities determines the range of cultivated plants. The presence of Johnson-grass however restricts it: according to the degree of infestation it decreases the number of plant species to be cultivated, and also their economy, partly because of yield losses, or by increasing of production costs (Kőrösmezei, 1994).

3. Physical methods of protection

3.1. Burning
This method is able to destroy the plant parts above the soil surface on cultivated plant free fields. The Johnson-grass can be restricted on a stubble by repeated burning, but nowadays this method is not recommended because of its natural protection drawbacks.
3.2. Inundation
The effect of inundation has been investigated by Mc Whorter (1972b). He found, that the rhyzomes did not die always even after a long-lasting inundation.

4. Chemical methods of protection
The protection possibilities by chemicals on an infested field will be determined basically by the cultivated plant.
4.1. Protection possibilities in case of the cultivation of a monocotyledone plant (maize).
4.1.1. Protection of seed-borne Johnson-grass.
The risk of chemical protection at using pre- or early postemergent usable product is, that to have an economically good effect the products need 10-20 mm precipitation following their spraying out. In the last years the choice of chemicals to be used in maize for basic treatment became wider (with pendimetalin, S-metolachlor, acetochlor, dimetenamid izoxaflutol) (Bihari, 2005). To investigate the effect of some preemergent usable chemicals (pendimetalin, S-metolachlor, acetochlor, dimetenamid izoxaflutol) on emerge of Johnson-grass the authors conducted a pot trial and a field trial in 2005 and 2006. Based on the results authors concluded, that all the four chemicals have a good effect against seedborn plantlets of Johnson-grass (Tóth and Lehoczky 2005, 2006). Even the modern newer active substance containing chemicals can not fully solve the problem of Johnson-grass just by a single treatment. In most cases – depending on the environment circumstances – a second treatment is necessary to kill the perennial, rhizome-born weed plants, eventually in another crop.

4.1.2.Weed control treatment in maize
The development of sulfonilurea containing products made possible the chemical protection against weeds in maize crops. These herbicides (nicosulphuron, rimsulphuron, tifensulphuron-metil) can be used up to the 7-8 leaves stage of the maize plants. For the effectivity of the products 1-3 leaf stage of the Johnson-grass is the best favourable, while in case of rhizome-born their height of 12-20 cm, at this stage are they most sensitive to the products. Extreme circumstances (temperature below 10°C or above 30°C, drought) caused stress and treatment of overdeveloped maize (8 or more leaves) increases the danger of phytotoxicity. Late treatment causes

damage of the maize plants, beside that the effectivity of the treatment is hindered also by shading of the weeds. Since the developmental stage of seedlings and that of rhyzome differs, moreover the emergence of the same forms may be long-lasting, we can only achieve the most proper treatment date nearest to the right weed development stage, if we use a share treatment (Tóth, 2006). Portitioning of adjuvants to the spray solution is necessary. If the product does not contain also surface-active component, its completion may not be neglected.

4.2. Treatments against rhyzomal Johnson-grass on stubble

This is the time period in which the systemic active substances quickest translocate. The most suitable active substance to be used against the Johnson-grass on a stubble is the gliphosate. Since it has a total effect it can be succesfully used against all perennial mono- and dicotiledons. The using of selective monocotiledone-killing products on stubble is economically not favourable, and also because of other problems not recommended, and not widely used.

4.3. Protection of Johnson-grass in dicotiledone cultures

A widely used protection practice on dicotiledone cultures is the basic treatments with soil herbicides against Johnson-grass seedlings, followed by a crop treatment against rhizomal plants, which herbicide however kills well also those plantlets which escaped or survived the basic treatment.

4.3.1. Basic treatment

To kill broad-leaf weeds exists a choice of different groups of herbicides. In the group of dinitroanilids mainly the trifluralin or benefin containig products have good effect. After spraying out they have to be mixed in the soil, soon after, or at the same time. These herbicides kill the emerging seedlings. Products of this group have a short active time, therefore it is important, to ensure favourable circumstances for emerging of mass of the seeds (loose, fine soil structure, proper soil humidity, and higher soil temperature). The group of imidazolines have equally good effect whether absorbed through leaves or soils. They can be used ppi (applied before sowing and mixed in the soil), presowing or postemerge, too. The best results can be achieved when sprayed onto 2-3 leaves weeds (early postemergent). Spraying their higher doses they have good effect on better developed seedlings or even rhizomal plants, but since they need a larger time to break down, less succeeding crops may follow their use.

4.3.2. Crop treatment

Johnson-grass and other rhizomal or stolony type difficult weed can be killed easier, cheaper and more effectively in broad-leave cultures than in a maize crop. There is a choice of special monocotiledone killer in circulation in the last years (the ariloxi-fenoxi-propionates). The most proper time of application is, when the Johnson-grass plants are 20-25 cm high. In this stage the foliage is big enough to absorb the necessary amount of herbicide to kill the plant, and also the plants are most sensitive at this stage. At the application also the fenological stage of the cultivated crop has to be taken into consideration. Crops with large shading surface may hinder also the effectivity of higher doses used. Spraying under the foliage may have a satisfactory effect even at later sprayings. The killing effect can considerably be increased by a proper application technology (using adjuvants, better droplet formation, proper choice of nozzles). An important condition for a good success is the choice of optimal time of spraying (optimal wheather). Most succesful treatment can be made in a whether following a rainy, warm, humide period, since the metabolism of the weeds is then most active.

Conclusions

Herbicides usable against the Johnson-grass have an effectivity above 90 %. No one product exist by which a field could be cleaned from Johnson-grass with a single treatment or even during a year. The basic of decision could be, whether we want to kill or restrain this weed in a shorter period, or how much is endangered the crop we intend to produce. Beside the detailed analysis of those practical stand-points, the cost-relations can be the basic of the decision (cost per hectares).

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POSSIBILITIES OF INTEGRATED WEED CONTROL AGAINST JOHNSON-GRASS (*SORGHUM HALEPENSE* /L./ PERS.)

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Summary

The Johnson-grass (*Sorghum halepense*) belongs to those weeds causing considerable damages in Hungary. According to Holm et al. (1977) Johnson-grass is the 6th most important weed worldwide. The extreme wheathers of the last years made clear, that not a single year or possibility may be omitted during the control of this weed. We have no chance to kill it without thorough knowing of the biology of this species. An important element of the protection is the hindering of the settlement of the Johnson-grass. If it is present on the field, the protection methods have to be coordinated. A new possibility to detect and identify infested field spots is the using of precision place determination instrument and method, which helps also to reduce considerable the amount of herbicide to be sprayed out.

STUDY OF PHYTOTOXICITY OF HERBICIDES ON GREEN PEA

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The size of pea crop area will be determined partly by the demands of canning and deep-freezing industry and partly by the demands on dry peas. In the last years the green pea crop area was between 20 to 30 thousand hectares in Hungary, and that of the pick pea area between 10 and 20 thousand hectares (Hornyák, 2005).

The pea crop is especially sensitive to the weed damage; therefore much attention has to be paid for its cultivation. The correct weed control helps the continuous development and ripening of the pea crop, makes possible to conduct an easier harvest and enhance preceding crop value, since it results in weedless stubble. The herbicides with different mode of biological action may have different effect on the life processes of the pea plant, they may hinder of its development, may cause damages. The plant may compensate these effects later on, but also may suffer of lasting damages leading to yield reduction. (Nádasy and Wágner, 2005).

Weed control in the pea crop may be done pre-sowing, pre-emergence or using post-emergence herbicides. In the green pea production mostly pre-emergence herbicides are used. The reason of its is that they kill weeds during the critical, early development stage of the pea plants, and put an end to the competition between cultivated plant and the weeds. Its successful application combined with agro technical and mechanical methods will make unnecessary later crop treatments (Nádasyné and Wágner, 2004).

The different pea cultivars differently tolerate the herbicides because of the thickness of their waxy layer. Using a new cultivar one has to get information on its sensitivity against the different herbicides (Kádár, 2005).

Benécsné (1994) tested 25 cultivars using 5 herbicides resp. herbicide combinations. She estimated the phytotoxicity using the 1 to 9 scores of the EWRC scale. In the case of Igran 500 FC and Bladex 50 SC she observed light phytotoxicity, the plants showed leaf yellowing, necrotic spots starting from the leaf lamina edges. The recommended dose of chloracetanilide group belonging propizochlor-containing Proponit 840 EC caused different toxicity on 80 % of the cultivars tested, while its provocative double dose caused high toxicity on all the tested cultivars. The Stomp 330 EC caused typical leaf necroses, and by this differently strong damage.

Singh and Wright (2002) studied effects of three herbicides in glasshouse conditions, using two pea cultivars, and investigated the effects of herbicides on node number, on the growing patterns of pea and on the yield. The double doses of Basagran (active ingredient: bentazon) caused chlorosis of the plants. The numbers of node were decreased mostly by the double doses of Basagran and Gesagard, and all herbicide decreased dry matter of the shoots and legumes.

The flumioxazine inhibits the growing of pea plant and causes leaf necroses, still do not decrease yield loss (Boydston et al,. 2002, Boydston, 2002). Al Khatib et al. (1999) used low-concentrated sulfonylurea products pre- and post-emergently and bentazon combined with detergent for weed control in green pea crop (Al Khatib et al., 1995).

Materials and Methods

Based on results of earlier glasshouse tests, we made a field experiment with four replications in spring 2006, using 5 herbicides of different mode of action. We tested their effect onto the growing of vegetative parts of the pea plants. Four of the products were pre-emergence and one post-emergence (Table 1.). Among them the Pledge 50 WP is not permitted in pea culture in Hungary yet.

Table 1. Herbicide treatments in the experiment

Herbicide	Ingredient	Single rate	Double rate	Mode of application
Afalon Dispersion	linuron	2 Lha^{-1}	4 Lha^{-1}	Pre
Command 48 EC	klomazon	0,2 Lha^{-1}	0,4 Lha^{-1}	Pre
Pledge 50 WP	flumioxazin	0,08 $kgha^{-1}$	0,16 $kgha^{-1}$	Pre
Sencor 70 WG	metribuzin	0,35 $kgha^{-1}$	0,7 $kgha^{-1}$	Pre
Basagran	bentazon	3 Lha^{-1}	$6Lha^{-1}$	Post

Two doses were used, the recommended one according to the permission document, and its double. The pre-emergence treatments were made two days following the sowing. The post-emergence herbicide was sprayed out two weeks following the emergence. The experiment took place on a brown forest soil of the training farm of the Pannon University. The cultivar used was the early ‘Karlos’.

To estimate the phytotoxicity we collected samples in two different dates, and made a visual observation. The first sampling was made at 2-3 leaf-stages, the second at flowering. Each of the samples contained 10 plants collected random from the plots, along a diagonal line of the plot. The lengths, fresh and dry weight of the shoots were determined.

Results

The Figure 1. shows the length of shoots following the herbicide treatments at the two samplings. At the first sampling the average length of the untreated plants was 14.4 cm. Recommended doses of Command 48 EC, Pledge 50 WP and Sencor 70 WG did not influence significantly the shoot length. Post-emergently used Basagran a little bit increased the length of shoot. Both concentrations of Afalon Dispersion significantly decreased shoot length: the recommended doses decreased the length more than 20%, while the double doses with 40%. Doses of Command 0.4 l/ha, Pledge 50 WP 0.16 kg/ha and Sencor 70 WG 0.7 kg/ha also inhibited shoot length.

At the second sampling the double dose of Command 48 EC, Pledge 50 WP and Sencor 70 WP significantly decreased shoot length. Both concentration of Afalon Dispersion significantly hindered the growth (10% and 35% respectively).

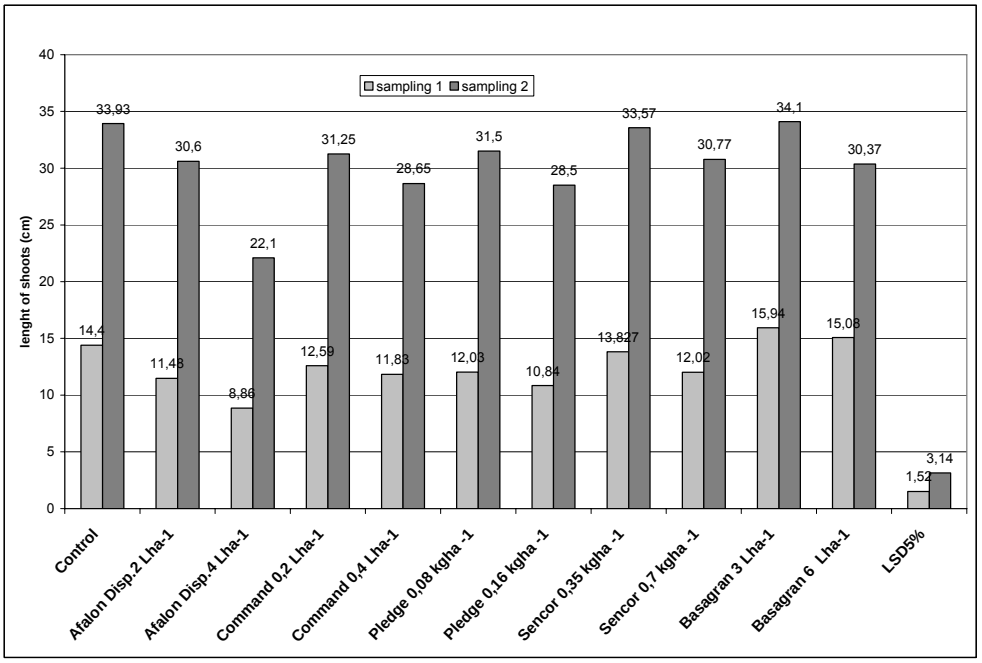

Figure 1. Effect of herbicides on length of green pea shoots

Figure 2 shows changes of the fresh weight of shoots: the treatments caused considerable differences. At the time of the first sampling a small decrease could be observed as an effect of recommended dose of Pledge 50 WP and double dose of Sencor 70 WP. The double concentration of Command 48 EC and Pledge 50 WP significantly decreased the fresh weight. Both doses of Afalon Dispersion caused a decrease, the double dose even 60%. For the Basagran treatment the plants reacted with a small fresh weight increase.

At the time of the second sampling the concentrations of Sencor 70 WP 0.35 l/ha and of Basagran 3 l/ha caused a little, but no significant increase. The other herbicides differently (2-57%) decreased the fresh weight of the shoots. A significant change could be observed in case of Command 48 EC 0.4 l/ha and Afalon Dispersion 4 l/ha doses. These herbicides decreased fresh weight 34% and 57% respectively.

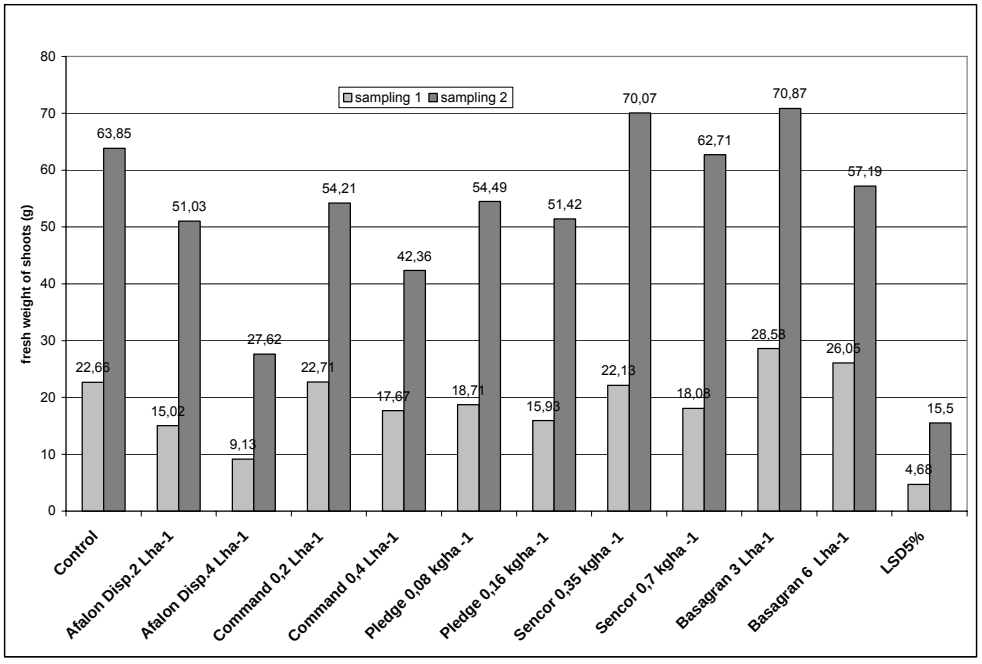

Figure 2. Effect of herbicides on fresh weight of green pea

Examination the effects of herbicides on the dry weight, it has been found, that the effects were very differing. At the first sampling the highest dry weight were measured in case of Basagran 3 l/ha, this significantly differs from that of the untreated plants. The recommended doses of Command 48 EC, Pledge 50 WP and Sencor 70 WG did not cause notable change, but their double doses significantly decreased the dry weight of the plants. Considerable decrease could be observed at both concentrations of

the Afalon Dispersion. In case of the recommended dose the dry weight decreased with 34%, and at the double dose with 64%.

Similar results were shown at the second sampling, too. The recommended dose of Basagran somewhat increased the dry weight, that of Sencor did not make a change, the other herbicides differently decreased the dry weight. The most severe decrease of the dry weight – 56,5 % - caused the double dose of Afalon Dispersion. This can be explained with the photosynthesis hindering effect of the linuron, which means consequently assimilation- and growing inhibition, too.

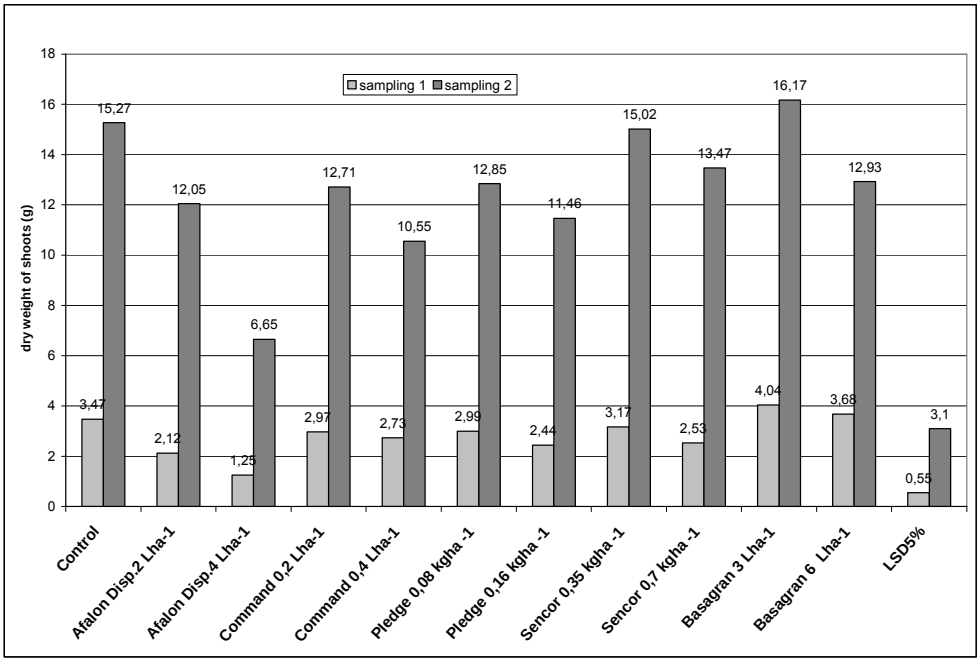

Figure 3. Effect of herbicides on dry weight of green pea

During the experiment, three weeks after emergence also visual observation were made to determine (Table 2.) the effects of phytotoxicity in percentage (Dancza, 2004). No symptoms of damage could be observed in case of Sencor 70 WG and Basagran treatments. After the treatment with Pledge 50 EC recommended dose very mild symptoms could be observed: light deformation and light dwarfing. The double concentration caused however small brown spots, leaf deformation, dwarfing, weaker growing in relation to the plants of the untreated plots. The recommended dose of Command 48 EC caused definite symptoms: first yellowing leaf spots at he borders, which became later on white, occupied the third, then the half of the leaf lamina. In the case of double dose the symptoms were more definite and there was also a light deformation.

The plants treated with Afalon Dispersion showed the most definite symptoms. Using the recommended dose the treatment caused chlorotic symptoms on the leaves, some plants emerged deformed, at further growing they were also deformed, and remained smaller than the others. Some seedling died because of the herbicide. The double dose caused definite yellowing, deformation and dwarfing. Many plants became white after emergence, wilted, drooped, dried out and died. To the end of the season more than 50% of the crop died out.

Table 2. Phytotoxic effect of herbicides on green pea

Treatments	Phytotoxic effect%	Characteristics
Afalon Dispersion 2 Lha^{-1}	25	strong damage
Afalon Dispersion 4 Lha^{-1}	50	heavy damage
Command 48 EC 0.2 Lha^{-1}	5	definite symptom
Command 48 EC 0.4 Lha^{-1}	5	definite symptom
Pledge 50 WP 0,08 $kgha^{-1}$	5	definite symptom
Pledge 50 WP 0.16$kgha^{-1}$	10	damaged
Sencor 70 WG 0.35 $kgha^{-1}$	1	very light symptom
Sencor 70 WG 0.7 $kgha^{-1}$	0	without symptom
Basagran 3 Lha^{-1}	0	without symptom
Basagran 6 Lha^{-1}	0	without symptom

Based on the results it can be established, that the linuron containing Afalon Dispersion damaged most the growing of the pea plants, causing severe phytotoxic symptoms. The double doses of Pledge 50 WP and Command 48 EC had also a damaging effect on the pea plants. The products Sencor 70 WP and Basagran did not cause symptoms on the pea cultivar tested in this field experiment.

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Summary

The pea crop is especially sensitive to the weed damage; therefore much attention has to be paid for its cultivation. The correct weed control helps the continuous development and ripening of the pea crop, makes possible to conduct an easier harvest.

We made a field experiment with four repetitions in spring 2006, using 5 herbicides of different mode of action. We tested their effect onto the growing of vegetative parts of the pea plants. Afalon Dispersion (linuron), Command 48 EC (klomazon), Pledge 50 WP (flumioxazin), Sencor 70 WG (metribuzin) was used pre-emergence and Basagran (bentazon) post-emergence. Among them the Pledge 50 WP is not permitted in pea culture in Hungary yet. To estimate the phytotoxicity we collected samples in two different dates, and made a visual observation. The first sampling was made at 2-3 leaf-stage, the second at flowering. Each of the samples contained 10 plants collected random from the plots. The length, fresh and dry weight of the shoots was determined.

Based on the results it can be stated, that the linuron containing Afalon Dispersion damaged most the growing of the pea plants, causing severe phytotoxic symptoms. The double doses of Pledge 50 WP and Command 48 EC had also a damaging effect on the pea plants.

MATCHING BACKPACK SPRAYER APPLICATION TECHNOLOGY TO AN ARRAY OF AGRICULTURAL PEST CONTROL PRODUCTS[4]

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Introduction

Small-scale horticultural farmers grow a diversity of crops requiring an array of products for pest control, most frequently applied as liquids. Backpack sprayer's are evaluated and reviewed in this study for application of pesticides by small-scale horticultural producers. A review of conventional pesticides and approved pest control products used in organic farming indicates significant deficiencies a) in many cases detailed application instructions are not provided by chemical manufacturers, only use rates; b) product formulations vary widely in viscosity and particle size. This study examines “deployable” resources for small scale farmers.

Literature

The application of liquid products to horticultural crops by small-scale agricultural producers is generally problematic in terms of accurate and cost-effective sprayer application systems. Examination of the issues reveals there is a disconnect between the companies that are involved in manufacturing the sprayer application components and products utilized by farmers. This project is designed particularly to address this specific need for small-scale horticultural crop farmers.

More specifically, there are companies that manufacture sprayers who usually do not supply an adequate range of accessories to apply the multitude of various products. There are companies that specialize in manufacturing sprayer components such as nozzles, screens, strainers, check valves, filters, specialized speedometers, etc. For instance, there are over 60 nozzle and strainer parts that fit a Solo model 475 backpack sprayer listed in TeeJet catalog #73 (Spraying Systems, Catalog #73). The Solo on-line catalog lists three choices and would be inadequate to handle an array of

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agricultural liquid application products (Solo Sprayers). The third type of company involved manufactures the array of liquid application products that address pest control. The three companies involved lack a "systems" approach to address the farmers' requirements. Farmers are left in a quandary of having to integrate the components on their own, adding to the already overburdening array of equipment and management issues.

Examination of the "directions for use" of crop protection/production liquid products in the OMRI approved list reveals everything from precise to vague application directions. The DiPel DF label has specific spray application directions while the OmegaGrow label provides limited application parameters (DiPel DF Crop Label, 2005; OmegaGrow Crop Label, 2005). Farmers are caught in the middle; they purchase a sprayer and products then make critical applications that can be the difference between success and failure. A recent publication by the New York Agricultural Experiment Station (Caldwell et al., 2006) addresses the use of OMRI listed products for insect and disease control. Details pertinent to spray applications are not thoroughly addressed. A personal communication with Rosen indicated the authors had an interest in further application technology information. Agricultural crop production requires "precise window of opportunity" applications based upon roadblocks such as wind, rain and field conditions. In order to meet the application timing criteria given the impediments, it is important that application equipment operates adequately so farmers are not stymied in their attempts to meet crop production goals. Clogged nozzles in the middle of an application can require an extraordinary amount of the farmer's time to rectify leading to application errors and lost productivity. This jeopardizes overall farm profitability, impacting the lifestyle of the family as well as leading to potential food safety issues for consumers.

Fortunately, appropriate spray equipment to handle the array of liquid agricultural products such as fish emulsions, powdered nutrients, insect, disease and weed killing products derived from soaps, oils, compost tees, etc. is available from a multitude of sources. Many small sprayers are manufactured in countries that utilize various standards such as metric and British standards making compatibility an issue. Farmers have to be prepared to deal with incompatibility issues of required spray components (nozzles, screens, pressure regulators, etc.) that may not be available locally. This project will train agricultural educators on the many variables related to products and equipment required by farmers to address the noted issues.

Materials and Methods

Ten different backpack sprayers representing four distinct operating characteristics were evaluated at a constant pressure and flow rate of 30 pounds per square inch operating pressure and a nozzle flow rate of 0.52 gallons per minute at the noted operating pressure. The four operating characteristics were represented as follows: 1) hand operated piston pump sprayers, 2) hand operated diaphragm pump sprayers, 3) electric motor powered diaphragm pump sprayer, 4) gasoline engine powered centrifugal pump sprayer. The hand powered piston pump and diaphragm pump sprayers noted above were also evaluated at 15 pounds per square inch operating pressure and a nozzle with a flow rate of 0.37 gallons per minute. Each sprayer was operated in order to produce 1 gallon of spray material and the time required in the number of hand strokes recorded (except for electric and gasoline powered sprayers).

Overall backpack sprayer performance evaluation was also determined by selecting for experienced backpack sprayer operators to apply spray material to four different vegetable crops including peppers, eggplant, tomatoes, and summer squash. These four crops represented leaf surface areas varying from “low” to “high” volumes. The greatest leaf area treated was mature summer squash and the least leaf area was peppers. 100 feet of row of each crop were treated by each person. They spray equipment operators were asked to independently evaluate each of the 10 sprayers with an overall rating from 1 to 10 with one equaling the worst performance and 10 equaling the best performance. Operators combined several factors including physical exertion, comfort and spray application characteristics. Each sprayer was equipped with a 30 pounds per square inch pressure regulator and a spray nozzle producing 0.52 gallons per minute. In addition, gasoline powered air assisted misting sprayer and a gasoline powered centrifugal pump sprayer and an electric motor powered sprayer were included in the evaluation. These sprayers were operated without the pressure regulating valve and the 0.52 nozzle. Original equipment nozzles were utilized.

Results and Discussion

Hand operated backpack sprayer's varied substantially in performance as noted in Table 1, when evaluated at equal volume outflow and constant pressure. A pressure regulating valve (CF valve) was utilized to control pressure. The CF valve does not operate at pressures below 30 pounds per square inch nor above 30 pounds per square inch as opposed to other types of pressure regulating devices that do not allow pressure to exceed the upper

set limit but still allow spray outflow at lower pressures. As can be noted in Tables 1 and 2 the time to apply 1 gallon of spray material varied substantially which should not have been the case with the pressure regulating valve. This variability can be accounted for by operator fatigue and slight errors in pressure regulating valve characteristics (but that was not the main cause of variability). In some of the hand operated sprayer's operator fatigue attempting to maintain operating characteristics became the limiting factor. The data in Tables 1 and 2 indicate that for the hand powered sprayers the piston pump models outperformed the diaphragm pump models likely due to pump chamber volume, with the larger pump chambers outperforming smaller pump chambers. Mechanical leveraging devices on some sprayers reduced human fatigue.

Table 1. Backpack sprayers with high (30 P.S.I.) pressure

NJAES – SNYDER RESEARCH FARM – BACKPACK SPRAYER
EVALUATION AT 30 P.S.I. WITH "06" NOZZLE (0.52 GPM)

SPRAYER MODEL	OPERATION POWER SOURCE	STROKES/ GAL.	RESIDUAL	TIME: REQUIRED
SOLO #475 DIAPHRAM PUMP	HAND	123	1 oz.	2 min., 3 sec.
SOLO #425 PISTON PUMP	HAND	88	4 oz.	1 min., 48 sec.
SHINDAIWA SP #415 PISTON PUMP	HAND	57	2.5 oz.	1 min., 57 sec.
MARUYAMA #409 PISTON PUMP	HAND	95	1 oz.	1 min., 52 sec.
HARDI BP #15 PISTON PUMP	HAND	108	1 oz.	2 min., 5 sec.
ECHO MS #100 DIAPHRAM PUMP	HAND	120	5 oz.	2 min. 9 sec.
STIHL SG #20 PISTON PUMP	HAND	96	4.5 oz.	1 min., 48 sec.
BIRCHMEIER IRIS 15K PISTON PUMP	HAND	120	2 oz.	1 min., 52 sec.
**SHURFLO SRS #600 (HIGH PSI SETTING)	ELECTRIC MOTOR	NA	NA	5 min., 38 sec.
**SHURFLO (O3 NOZZLE) (HIGH PSI SETTING)	ELECTRIC MOTOR	NA	NA	3 min., 58 sec.
ECHO SHR #210	Gasoline	NA	NA	2 min.

OPERATION PARAMETERS: All sprayers were operated with a CF 30 psi pressure regulator valve (except Echo SHR 210)
** Shurflo Electric Pump Pulses Conflicted With CF Pressure Regulator

Table 2. Backpack sprayers with low (15 P.S.I.) pressure

NJAES – SNYDER RESEARCH FARM – BACKPACK SPRAYER EVALUATION AT 15 P.S.I. WITH "06" NOZZLE (0.37 GPM)

SPRAYER MODEL	OPERATION POWER SOURCE	STROKES/ GAL.	RESIDUAL	TIME: REQUIRED
SOLO #475 DIAPHRAM PUMP	HAND	136	1.5 oz.	2 min., 28 sec.
SOLO #425 PISTON PUMP	HAND	124	10 oz.	2 min., 32 sec.
SHINDAIWA SP #415 PISTON PUMP	HAND	72	9 oz.	2 min., 16 sec.
MARUYAMA #409 PISTON PUMP	HAND	124	8 oz.	2 min., 32 sec.
HARDI BP #15 PISTON PUMP	HAND	99	4 oz.	2 min., 0 sec.
ECHO MS #100 DIAPHRAM PUMP	HAND	124	9 oz.	2 min. 36 sec.
STIHL SG #20 PISTON PUMP	HAND	128	11 oz.	2 min., 32 sec.
BIRCHMEIER IRIS 15K PISTON PUMP	HAND	148	6 oz.	2 min., 40 sec.

OPERATION PARAMETERS: All sprayers were operated with a CF 15 psi pressure regulator valve (except Echo SHR 210)
** Shurflo Electric Pump Pulses Conflicted With CF Pressure Regulator

In the Table 3 the average value of sprayer performance assigned by the four individuals supports the points noted above comparing hand operated piston pump sprayers to diaphragm pump designs. The gasoline powered misting sprayer and the gasoline powered pump sprayer rated highly in operator performance but generally equal to the best of the hand powered piston pump sprayers. The electric motor powered diaphragm pump sprayer provided medium performance in the comparison ratings. This is a relatively new design and approach to backpack sprayer design.

The overall cost of each sprayer as purchased in the United States in US dollars is listed in the Table 4. Considering sprayer cleanup and multiple uses the cost and performance of the sprayer should be carefully weighed. Several lower-cost sprayers can be purchased for the price of some of the higher cost units. Farmers growing several different crops on a small scale may benefit substantially in time savings by maintaining several backpack sprayer's each utilized for separate operations such as the application of herbicides, insecticides and fungicides reducing the potential for crop injury.

Table 3. Backpack sprayers' performance

SPRAYER PERFORMANCE EVALUATION

* 1 = WORST
10 = BEST

SPRAYER MODEL	#1 OPERATOR	#2 OPERATOR	#3 OPERATOR	#4 OPERATOR	AVERAGE RATING
SHINDAIWA SP #415 HAND PISTON PUMP	8	9	7	7	7.75
MARUYAMA #409 HAND PISTON PUMP	2	2	2	5	2.75
ECHO MS #100 HAND DIAPHRAM PUMP	3	3	4	8	4.5
SOLO #475 HAND DIAPHRAM PUMP	4	6	4	7	5.25
SOLO #425 HAND PISTON PUMP	6	7	5	7	6.25
HARDI BP #15 HAND PISTON PUMP	3	3	2	6	3.5
STIHL SG #20 HAND PISTON PUMP	4	6	3	6	4.75
BIRCHMEIER IRIS 15K HAND PISTON PUMP	3	5	2	5	3.75
ECHO GAS MOTORIZED SHR #210	6	8	5	8	6.75
STIHL GAS MISTBLOWER SR #420	7	9	7	8	7.75
**SHURFLO SRS #600 (HIGH PSI SETTING)	5	5	7	5	5.5

* 4 Experienced individuals with backpack sprayers utilized sprayers in a field of several vegetable crops; peppers, eggplant, tomatoes and squash for comfort, performance and fatigue

Table 4. The prices of backpack sprayers

Backpack Sprayer Prices

Company	Model	Purchase Price	Website
BIRCHMEIER	15k	$221.00	www.birchmeier.com
	BCS (closed Sys.)	$245.00	
Echo	SHR-210	$523.00	www.echo-usa.com
	MS-100	$130.00	
Hardi	BP-15	$200.00	www.hardi-us.com
Maruyama	M-409	$65.00	www.maruyama-us.com
RL Proflo	614RL (4 gal.)	$80.00	http://rlflomaster.com
Shindaiwa	SP-518	$106.00	www.shindaiwa.com
	SP-415	$100.00	
Shurflow	SRS-600	$190.00	www.shurflow.com
Solo	425 (Piston)	$90.00	www.solousa.com
	475 (Diaphram)	$90.00	
Stihl	SR 420	$540.00	www.stihlusa.com
	SG 20	$100.00	

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Spraying Systems. TeeJet Spray Products, Catalog #73. PO Box 7900, Wheaton, IL.

AN INTERREG PROJECT FOR THE EFFECTIVE AND SAFE PLANT PROTECTION IN THE EU

László Radócz – György J. Kövics – István Szarukán

Public Utility Foundation for Development of Plant Protection Teaching (NOFKA), Debrecen, Hungary

The aim of this project is to develop regional, post-gradual plant protectional courses and connecting extension services (databases) in the areas of Hajdú-Bihar county (Hungary) and Bihor county (Romania) with locations of Debrecen and Oradea (mirror projects). The programme provides special post-gradual courses in the field of plant protection for farmers in the region (25-25 persons in Debrecen and in Oradea) and can act as models for further developments. In these projects EU-related curriculum as well as handbooks, power point presentations (in Hungarian and Romanian languages) planned to be developed in close collaboration. Professional training books and CD-ROMs will be purchased to develop the educational infrastructure. The results of the projects and continuous professional education are not only more precise pesticide application or less pesticide use in the agricultural sector but more healthy (and human safe) agricultural products and positive impact on environmental-pollution parameters. The information will be provided via fax, bulletins and special WEB-pages for the target groups. At the end of the projects final evaluation conferences (in Hungary and in Romania) will be held for the end-users.

The number of the project: INTERREG III/A HU-RO-SCG-1/329

The duration of the project: 28.02.2006-31.03.2007.

Areas of the project activity is covered:

- Human resource development with special courses for Hungarian and Romanian agricultural producers.
- Economical development in agricultural production systems based on post-gradual courses and extension services.
- Protection of the environment and consumers by up-to-date knowledge based, more precise or reduced pesticide application.
- Development of institutional cooperation in creating special up-to-date WEB sites, databases between the partners.

Project Locations: Hungary, Hajdú-Bihar county, Debrecen Romania, Bihor county, Oradea

Amount requested from the Contracting Authority:

Total eligible cost of the action	Amount requested from the Contracting Authority in this proposal	% of total cost of action
12.512.600 HUF	11.883.216 HUF	94.97%

SIGNIFICANCE OF PESTICIDES IN THE INTEGRATED PLANT PROTECTION

Quantitative and Qualitative Characteristics of the Pesticide Usage

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Introduction

The continuous increase of the population on the Earth makes the production of more food necessary. The per capita arable area decreased substantially, the world average is about 0.1 ha. Among the available natural sources of energy soil has a determining role with respect to food production. Soil is a conditionally renewable natural resource, therefore the maintenance and enhancement of its fertility is of great importance.

During the food production and its storage the different pests may cause considerable losses, and damage. The amount of losses caused by living organisms comes to 35% on average worldwide. That is, why plant protection experts have such a highly responsible mission.

The materials, tools and technologies used during food production have an influence on the quality of the products and their processed forms. A number of different methods (agrotechnical, physical, mechanical, biological, chemical and integrated) are available, their use and effect may be very different.

The need for a chemical plant protection has an old history. The first chemicals were arsenic, quicksilver, copper salts, elementary sulphur and plant extracts like nicotine, pirethrum and neem. The research on plant protecting chemicals and their development has got big success in the 1960s (Table 1).

Table 1. **Number of plant protection products in Hungary** (Source: Növényvédő szerek és termésnövelő anyagok /Pesticides and regulators 2006/)

Years	Product	Active ingredient
1960	50	-
1966	238	-
1974	256	99
1978	334	173
1985	488	227
1990	613	278
1999	750	330
2002	823	337
2006	795	281

Pesticides played an important role in the development of the agricultural production, the high yielding plant production systems with their high yields unimaginable earlier could not be developed. Industrialized food production does not belong to those environment saving procedures which could meet the requirements of the principle of sustainability. The rate and costs of the artificially introduced inputs of the production are too high.

Materials and Methods

The author based his investigations on using the statistical data issued by the European Union and Hungary regarding the pesticid usage, quantities and quality.

Results

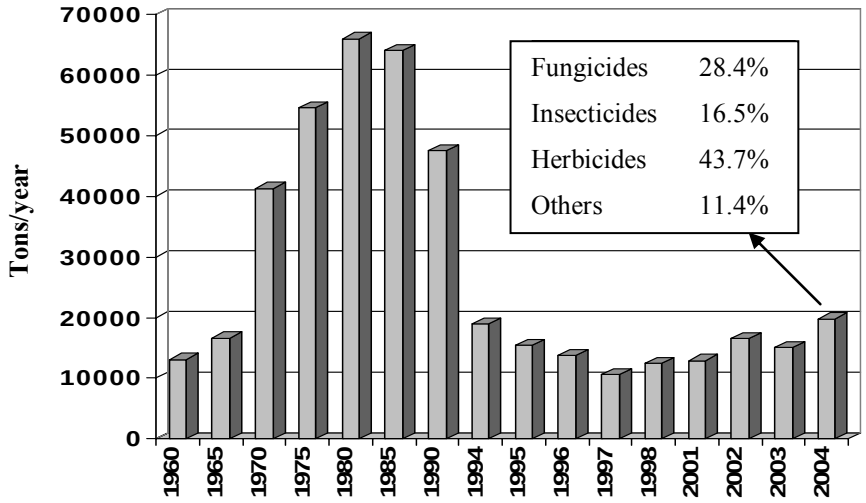
The aim of the farmers was to achieve the highest yields, both in Europe and in Hungary in the 1970s. For the 1980s they realized, that the economical effectivity and profit is not determined only by the highest yields. High rate of inputs was typical, and in Hungary, with its large fields it was not possible to take the local differences and their special demands into consideration.

Increasing food production have its restrictions, too. The biodinamical, ecological and integrated farming system, and within these the integrated plant production system can only comply with the requirements of sustainable development (Ángyán and Menyhért, 1997).

The wide range and, big amount of pesticides used in the last decades (Figure1) and realizing its effects on the environment draw the attention to the fact that other methods of plant protection have to be used.

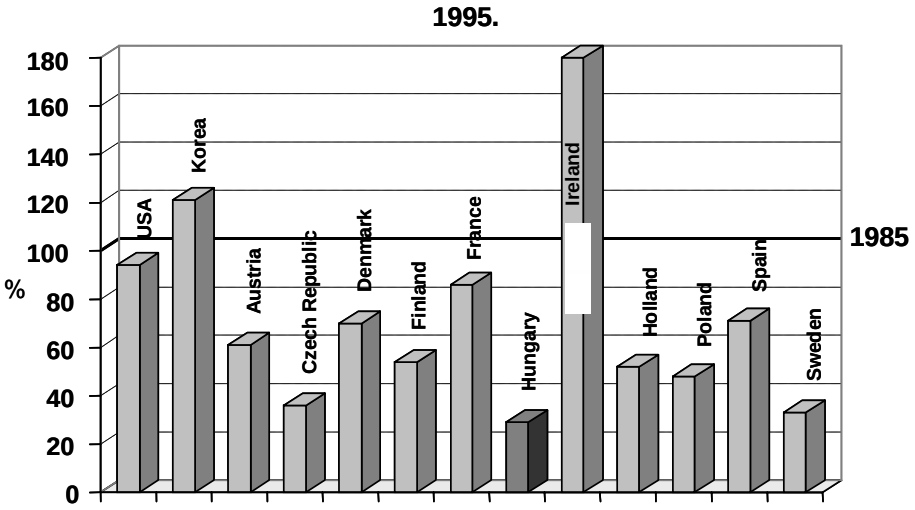
In Hungary, in the 1990s, the amount of pesticides considerably decreased, this is also shown by the data of Figure 1. The background of it was not only ecological (changes in the proprietorship, increasing prices), but also the fact, that in the meantime new, ultra low volume and especially effective pesticides (for example sulphonilcarbamide herbicides, pirethroids) were developed, and used throughout the country.

Figure 1. Pesticide usage in Hungary **(**Source: AKII)



The member countries of the European Union have drawn up the document „Toward sustainability". This document circumstantially analyses the environmental effects of industry, energy utilization, traffic, agriculture and of tourism. In the case of the agriculture, the considerable decrease of pesticide use and the application of integrated plant protection procedures were assigned as a direct target. (Lehoczky, 1999).

Figure 2. Pesticide usage of some countries in 1995 in relation to 1985 (100%) (Source: OECD Environment Data, 1997)



The amount of pesticides used in Hungary shows a favourable picture in comparison with international data (Figure 2). Since the time of the intensive chemical use of the 1980s, the amount of pesticides used has fallen back to about its quarter for the 1990s (Lehoczky, 2003).

For the measurement of environment pollution in a country the amount of pesticide given for an area unit is important data. This will be shown in Table 2 for the year 2002. According to this Portugal, Belgium and The Netherlands belong to the group of countries, who use the highest amount of pesticides. In Austria however pesticide usage has decreased considerably. (Figure 2).

In Austria more than 95% of the farms joined the announced agrar-environment-saving programs on the arable land and gardening, and by this they enjoy and have the highest amount of assistence per hectare (Pálmai, 2003).

The pesticide usage of Hungary is considerably less than that of the average of the European Union, which is in concordance with the EU proposals, and it is favourable. According to the present comprehensions the sustainable development should be followed, which can meet the present demands as it does not hinder the interests of the future generations. A part of this is the integrated plant production, including integrated plant protection.

Sustainable agriculture intends to use the modern, effective, environment saving plant protection methods, considering all the diversities and specialities of the environment. (Kuroli, 1999).

Integrated plant protection is an element of the integrated plant production, which is a sustainable way of farming. It means a complex farming system, which includes profitable plant production taking not only all local relations of soil, climate and economy into consideration, but also the environment. This system preserves the natural resources. By its application waste formation can be decreased, energy utilization can be improved, and environment pollution is minimized.

Table 2. Pesticide usage in Europe and Hungary, in 2000 (Source: Eurostat, KSH)

Country	***active substance metric tons***	***usage kg/ha***
France	94693	3.4
Spain	38027	1.5
Germany	28010	1.6
United Kingdom	18231	1.1
Italy	46068	3.2
Irland	1518	0.4
Portugal	24868	6.3
Greece	11131	0.9
Austria	3193	0.9
Sweden	1624	0.5
Danmark	2802	1.0
Finland	1157	0.5
The Netherlands	9707	4.9
Belgium	5425	4.1
European Union	286454	2.3
Hungary	8798	1.5

Integrated plant protection system is not a strictly determined method, but a system, using the newest scientific results, technologies, recommendations and experiences and uses all these to prevent and moderate eventual damage. Realizing integrated plant protection results in the unanbiguous decrease of chemical protection, but at the same time the chemical method still remains a determining part of plant protection (Gáborjányi et al., 1995).

In terms of the law (2000. XXXV) „Integrated plant protection is rational combination of possibilities of effective methods (plant sanitary, agrotechnical, physical, biological, chemical) which take care of the environment and especially of natural antagonists of pests".

Integrated protection needs the integration of professional factual knowledge and also of its high-level cultivation.

In Hungary, special education conditions concerns to the trade, buying and using of pesticides. According to a decree of the Ministry of Agriculture and Country Development (5/2001. (I. 16), farmers without proper plant

protection education are allowed to buy pesticides if they have a prescription from a higher educated expert, and chemicals, belonging to the I. and II. categories of circulation are allowed to be used only under the supervision of a plant protection engineer.

In Hungary pesticides are classified into three categories. Pesticides of the first category may only be used by higher qualified experts (with university or high school certificate), the pesticides of the 2nd category can be used by experts educated in a secondary school for plant protection (at least 80-hour-course on chemical plant protection), and those of the 3rd category belong to the freely usable pesticides.

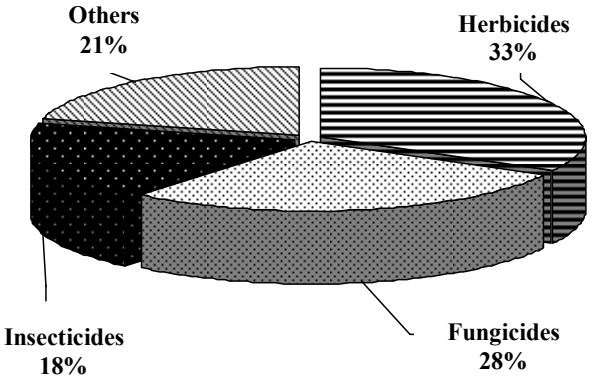
From Table 3 it can be seen, that in Hungary the usage of more than half of the pesticides (58%) is bound to qualification.

Table 3. Number and categories of authorized pesticides, 2006

	Trade categories			
	I.	II.	III.	Total
Herbicides	162	56	44	262
Fungicides	18	86	122	226
Insecticides	25	46	69	140
Others	56	11	100	167
Total	**261**	**199**	**335**	**795**

The distribution of pesticides according to their biological effects will be shown on Figure 3.

Figure 3. Distribution of pesticides according to their biological effect, 2006 (Source: Engedélyezett növényvédő szerek, termésnövelő anyagok /Pesticides and regulators 2006/)



The full amount of pesticides sold in Hungary in 2004 was 19880 metric tons, among them herbicides were the biggest part (44%), followed by

fungicides (28 %), insecticides (16.5%), while other chemicals amounted to 11.5% (AKI, 2005).

The application of pesticides ensures a more effective agricultural production, higher yields, but at the same time there is a risk of environment pollution, mainly soil and surface water pollution.

As a part of the Information and Monitoring System of Environment Saving (KIM), in 1992 a subsystem, the Information and Monitoring System for Soil Protection (TIM) was organized. The TIM has 800 sampling areas in Hungary representing all the arable fields, and in 1993 samples were taken from 106 places, from the three upper genetical layers of the soil, and tested. In 1996 and 1997 further 130 samples were examined for pesticide rests. In 1993 and 1996 the determination was made to see phenoxi-acetic-acid and triazine content, in 1997 also the phosphorus acid-esters and carbamates as well.

In 1996-97 as much as 130 soil samples were tested, 7150 measurings were made, and in 4.6% of the measurings showed pesticide rests, while higher value than the planned limit was found only in 5 cases, that is 0.07% of all the measurings. This result shows, that the Hungarian soils are practically free from pesticide pollution.

The active ingredients of the pesticides differ in their way of effectivity, in their chemical construction, but also in the time and method of using and technics, and they have different effects on the environment. Their presence, persistence, their metabolism and degradation may cause different biological reactions in the biotops, living organisms, and may cause differently severe environment pollution.

The high-level plant protection education has a three-decade long tradition in Hungary. The number of experts is almost 3000, who possess the high-level knowledge needed for the effective realization of integrated plant protection, and who acquire the new knowledge in obligatory courses.

The integrated plant protection system also applies the natural biotic regulation factors of the agrobiocoenosis to decrease the number of pest individuals under the economically acceptable level. This approach requires the consideration of the fact, that in case of every plant protection activity, especially of chemical usage, that beside pests, useful living organisms are also active in the same time and place.

In the integrated environment saving technology those products are allowed to use, which comply with the requirements of the European Union prescriptions (IOBC/WPRS, ISHS) and also of the national requirements. The detailed regulations of claiming of agricultural environmental management assistence is described in the 150/2004. (X.12) decree of the Ministry of Agriculture and Rural Development. The appendix of its modification decree 20/2006 (III.7.) FVM reviews in details the products

allowed or prohibited to use in the different crops in the agricultural environmental management program.

Nowadays it has become clear, that an average treatment (the same treatment) of a production unit or a large field may result in an uneven technology, since the soils, the fertilizer distribution, weed colonization, relief and microclimate are uneven, that means, that even within a field the plant protecting products and fertilizers has specifically to be given.

For the future precision agricultural methods have to be worked out. This is in progress, specialists and scientists are working on its possibilities cooperatively, who use modern place localization instruments (DGPS), and geographic information systems. The elaboration and application of precision methods of plant protection could considerable contribute to environment saving and economical pesticide use, and could give new possibilities to lower the amount of pesticides used in practice.

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SIGNIFICANCE OF PESTICIDES IN THE INTEGRATED PLANT PROTECTION

Quantitative and qualitative characteristics of the pesticide usage

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Summary

As a consequence of technical and scientific development of agriculture, the number of authorized pesticides has increased 15-fold since 1960 (now near to 800), and the number of active ingredients takes near to 300. Sustainable agriculture demands experts to choose and use from the wide choice of pesticides and active ingredients which least pollute the environment. This concerns to choosing the technological method, to the pesticides, to their dose, as well as to the technological discipline. In Hungary the situation is favourable with respect to the active substance amount per hectare, that is 1.5 kg, while that amounts to 2.3 kg/ha on the average in the European Union. This however does not mean, that experts have nothing to do for the decrease of environment pollution. Integrated plant protection, the special spraying technology using precision plant protection and by this the skilled use of pesticides have a determining importance in the maintenance of public health, in saving of environment, and last, but not least also in the economy. The test results of Information and Monitoring System for Soil Protection show, that the Hungarian soils are free from polluting pesticide residues.

It is important to keep this situation, which can also be helped by the place specific plant protection.

BIOLOGICAL TEST EXPERIMENTS ON MODELING EFFECT OF PESTICIDE DECOMPOSITION PRODUCTS

Diána Virág – Zoltán Naár – Attila Kiss

Károly Eszterházy College, Eger, Hungary

Herbicides sprayed mostly on the soil surface are exposed to the destruction by UV of the sun light. The toxicity of degradation products to the soil microorganisms is mainly unexplored. The growth inhibitory effect of the basic compound of carbendazim, acetochlor, simazine, chlorpyriphos and EPTC was compared with their photodegradation products (254 nm, 15 W). Common soil microbes, three bacteria (*Bacillus subtilis, Pseudomonas fluorescens, Mycobacterium phlei*) and three filamentous fungi (*Fusarium oxysporum, Penicillium expansum, Trichoderma harzianum*) were applied as test organisms. The antifungal effect of carbendazim significantly decreased as a function of UV-treatment time. However, the last remnant after the total elimination of basic compound markedly inhibited the growth of *T. harzianum*. The antibacterial activity of acetochlor degradation mix gradually increased, the transition products showed moderate antifungal activity, too. The degradation products of simazine were strongly hindered the proliferation of *M. phlei*; however, the test fungi were not sensitive to these compounds. Products of various antimicrobial spectrum appeared during the degradation of chlorpyriphos. The marked antibacterial effect of EPTC rapidly disappeared due to UV-light, but the end-product had strong and specific inhibitory effect against *P. expansum*.